11 12 76

Volume 95, Number 1, July 1976

British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

British Journal of Dermatology

EDITOR

DR R. H. CHAMPION
Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

DR J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London
DR RONALD MARKS, Cardiff
DR P.D.MIER, Nijmegen
DR J.N.S.MITCHELL, Royal Air Force
DR W.C.NOBLE, London
DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS

DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 U.K. (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

P, 25, 466

Printed in Great Britain at the Alden Press, Oxford

CUK-H06119-8-P25466

IMPORTANT NEW PRODUCT FOR PSORIASIS

Dithranol
olan

Much easier to apply, far easier to remove, and actually more effective.

DITHROLAN is quite simply the most convenient and effective form of dithranol in the treatment of psoriasis.

A careful and painstaking study carried out over the last 5 years at the Beaumont Hospital, Lancaster, demonstrated that a newly developed formulation of dithranol, now introduced as DITHROLAN, was not only very much easier to use than traditional Dithranol Paste, but was actually more effective[1, 2].

DITHROLAN adheres easily to the skin and can be applied quickly and conveniently. Previous applications of DITHROLAN can be removed simply by bathing – obviating the need for oil. As a result, patients actually carry out their treatment.

Dermatologists will appreciate DITHROLAN because of the considerable saving in treatment effort and time, and because of the excellent results obtained.

Presentation: DITHROLAN – containing 0·5% Dithranol B.P, 0·5% Salicylic Acid B.P. 90 gram amber glass jar. PL 0173/0024

References: 1. Brit. J. Derm. **93,** *205, 1975.*
2. Dermatology Digest **Feb,** *20, 1976.*

Further information and literature available from:

Dermal Laboratories Limited,
Tatmore Place, Gosmore, Hitchin,
Herts, SG4 7QR, England

Dithrolan

"I wish to apologise, both on my own behalf and that of Brocades, for doing the medical profession a disservice. We failed to make you fully aware of the effectiveness and clinical versatility of Pimafucin 2% cream as a topical treatment for fungal skin infections due to yeasts or dermatophytes.

I have no one to blame but myself. There has been ample opportunity to spread the word since 1967 when Pimafucin was introduced. The fact is I have kept our Medical Representatives too busy informing you about Locoid, De-Nol, Cyclospasmol 400 and other products to mention Pimafucin cream – except, perhaps, for use as an adjunct to Pimafucin vaginal tablets in the treatment of vulvo vaginitis.

Yet, as everybody here has known all along, there is a wealth of clinical evidence to support the efficacy of Pimafucin 2% cream. International investigations, embracing a wide variety of fungal skin infections, have consistently demonstrated impressively high cure rates.[1] In a recent British study[2] a 98% cure rate was achieved in 43 patients with culturally proven genital candidosis.

Fortunately for us, an increasing number of doctors have discovered the value of Pimafucin cream in dermatology by themselves, but even more patients might have benefited had we informed you about it in greater detail.

This announcement humbly and contritely seeks to remedy the situation.

Dare I suggest that just because I overlooked Pimafucin cream is no reason for you to do the same?"

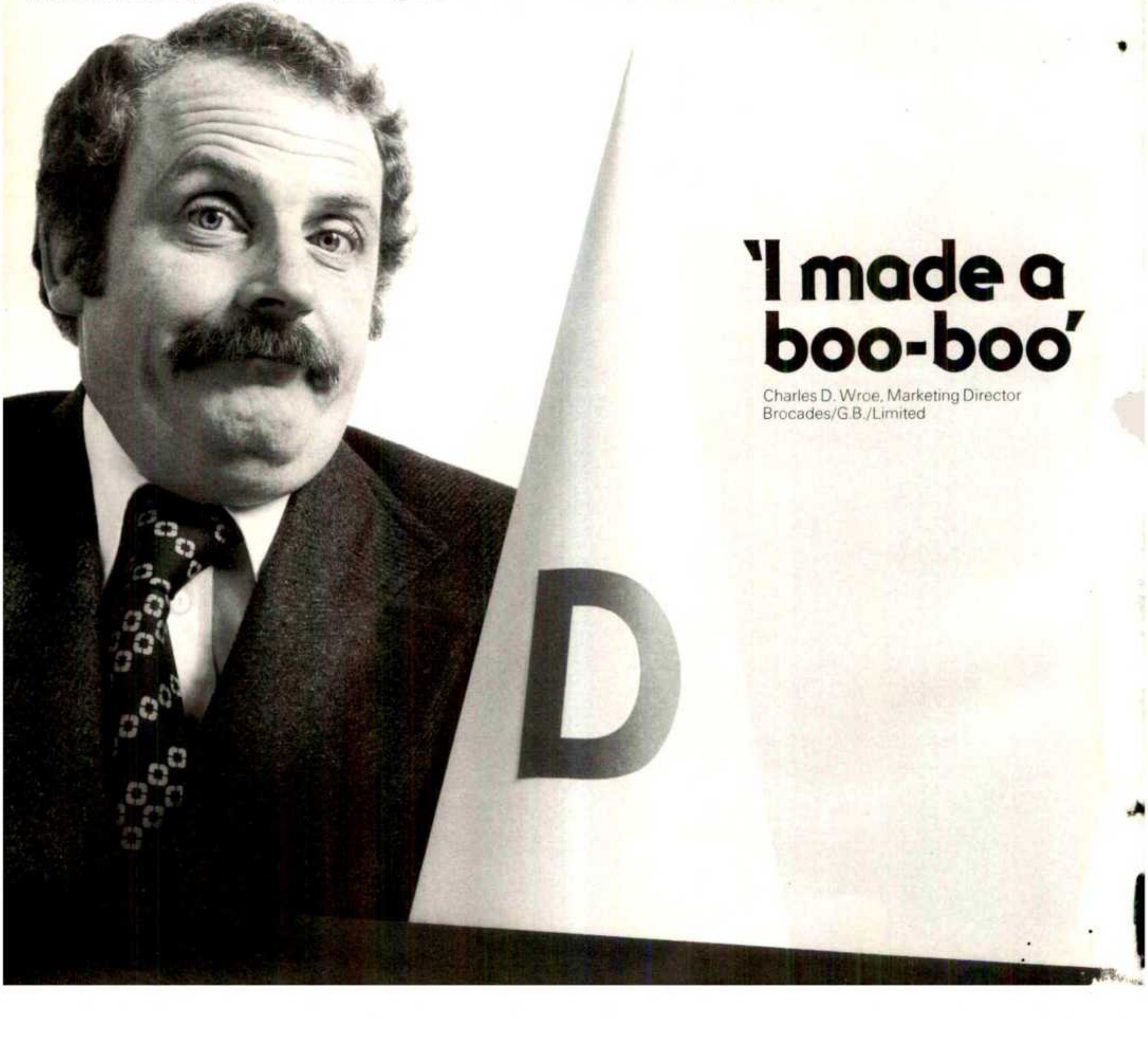
Pimafucin is the brand name for products containing natamycin and is available as 2% cream, vaginal tablets (for candidal, trichomonal and mixed vaginitis), 1% suspension (for oral candidosis) and 2½% suspension (for pulmonary fungal infections).

1. Raab, W.P. "Natamycin (Pimaricin). Its Properties and Possibilities in Medicine (1972)."
2. Masterton G. et al. Brit. Journal of Venereal Diseases (1975) Vol. 51 210.

pimafucin
cream
25 g 2% natamycin
Brocades (Great Britain) Ltd
Use as directed by the physician
pimafucin
cream

the cream of the broad spectrum antifungal creams.

For further information, please contact:
Brocades/Great Britain/Limited, Brocades House,
West Byfleet, Surrey. Telephone: Byfleet 45536.



'I made a boo-boo'

Charles D. Wroe, Marketing Director
Brocades/G.B./Limited

'OUTLOOK IN PSORIASIS'

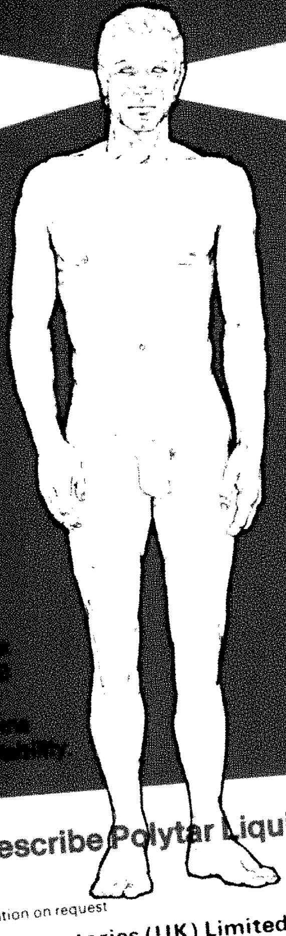
'The time-honoured applications of tar or dithranol and exposure to ultra-violet light remain the basis for local treatment'

Editorial, Brit. med. J. 1974,4,365

Polytar Liquid Psoriasis of the scalp

In a recent [illegible] hospital study, Polytar Liquid was compared with a standard [illegible] in 50 patients with psoriasis of the scalp. Polytar Liquid proved [illegible] in the removal of scale and the relief of itchiness. Patient acceptability was excellent.

Polytar Liquid combines effective control with patient acceptability



Polytar Emollient Psoriasis of the body

[illegible] acceptability.

"Polytar Emollient has a useful part to play in the treatment of psoriasis, particularly in out-patient treatment"

[illegible]

Polytar Emollient superior to liquor picis carbonis

For psoriasis – prescribe Polytar Liquid and Polytar Emollient

Full information on request

Stiefel Laboratories (UK) Limited 825 Yeovil Road, Slough, SL1 4JA

She's been to the chemis
she's been to her doctor
now she's come to you

In acne "... not all patien
respond satisfactorily [to
tetracycline] and there is
a need for more effective
or alternative therapy."

SEPTRIN* "... has been
clinically effective
in patients who did not
respond to long-term
tetracycline."

Brit J Derm. (1971), 84, 366.

SEPTRI
FOR ACN

Wellcome

Septrin contains trimethoprim and
sulphamethoxazole. Full prescribing
information is available on request.
Wellcome Medical Division
The Wellcome Foundation Ltd.
Berkhamsted, Herts

*Trade Mark

Molivate

(clobetasone butyrate)

Molivate is an ideal corticosteroid for childhood eczemas – such as atopic eczema – because it has been shown to be significantly more effective than hydrocortisone, yet to have the *same order of safety*.*

Cream – particularly suitable for the wet and weeping lesion.
Ointment – for the dry lichenified lesion.

Molivate* *British Medical Journal* (1975) **3, 626

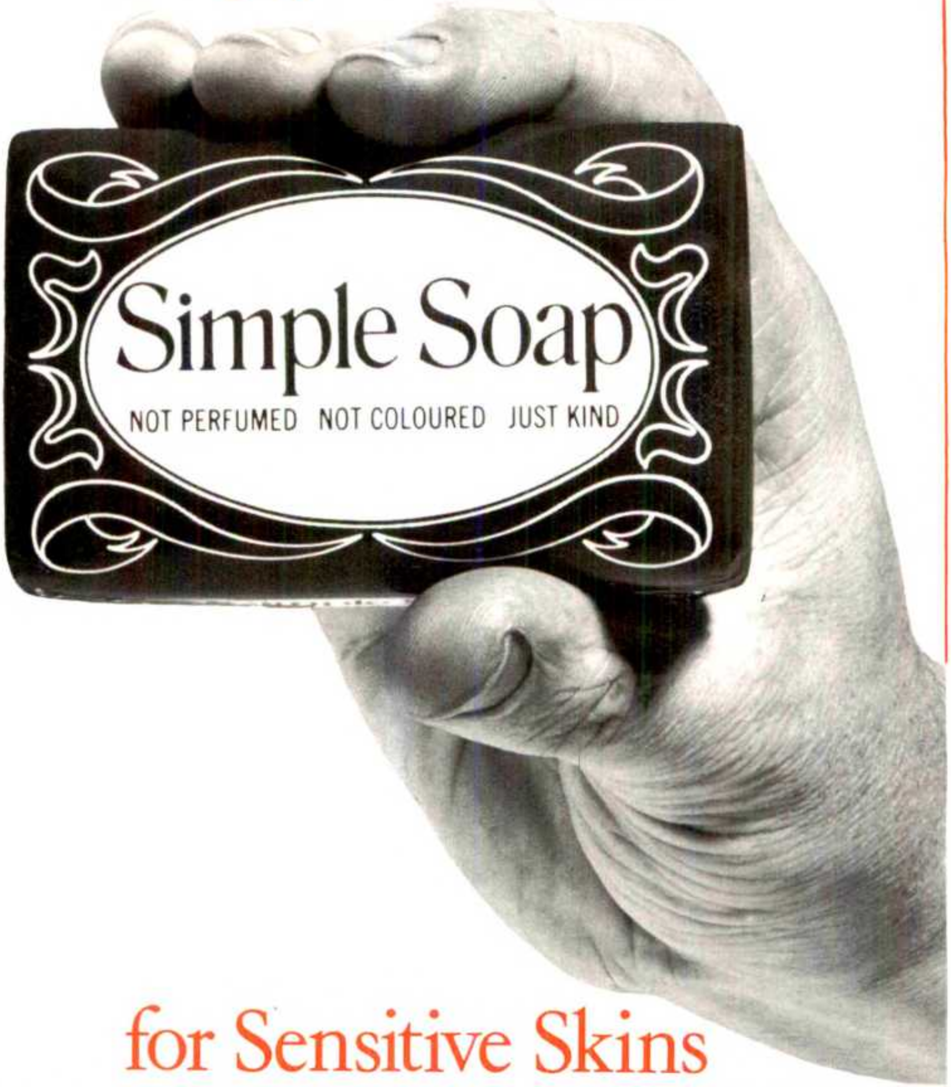
Molivate is available in 25 and 100 gram tubes

Glaxo

Full prescribing information on Molivate (trade mark) is available from Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE

When there is potential danger of skin conditions recurring or being exacerbated through irritation or allergic reaction……

Simply recommend



for Sensitive Skins

Skin problems may often be exacerbated by the colourants, perfumes or high-lather detergents contained in many brands of 'cosmetic' soap. Simple Soap contains none of these potentially harmful additives—it simply cleanses with the gentle lathering action of pure soap. That's why Simple Soap is widely used by hospitals throughout the country.

Simple Soap is available to your patients at chemists everywhere, for about 14p and 24p.

A regular supply of professional samples may be obtained on application to:
The Medical Division, Simple Soap Ltd., Hampton, Middlesex.

'The responsible carcinogenic
wavelengths are probably located
in the short sunburn waveband
around 300nm'*

SPECTRABAN

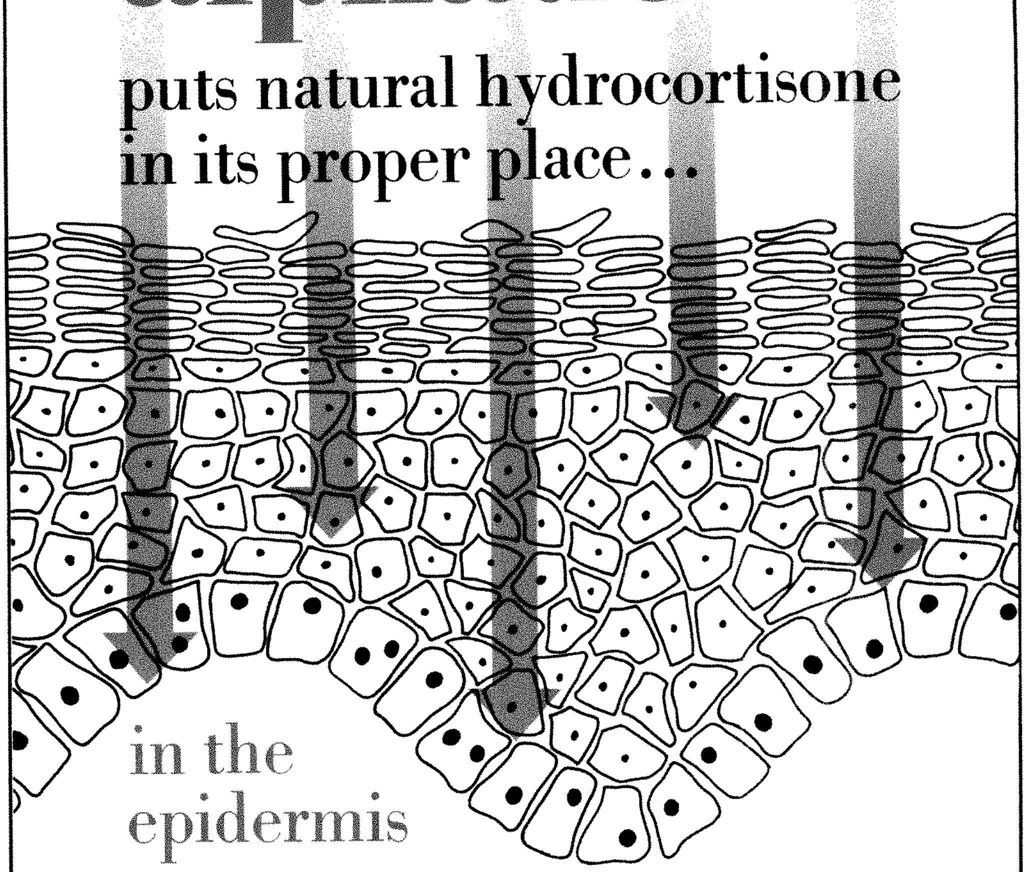
Provides complete protection
against solar radiation
within the range
290-320nm

**Spectraban protects
sun-sensitive skins**

Spectraban: isoamyl – p – N. N – dimethylaminobenzoate 2.5% in ethyl alcohol.
Packs: 60ml bottles

* Extract from recorded lecture 'The Sun and the Skin' by a Consultant Dermatologist.

Full information on request
Stiefel Laboratories (UK) Limited 825 Yeovil Road, Slough, SL1 4JA



alphaderm

puts natural hydrocortisone in its proper place...

in the epidermis

Alphaderm's unique drug delivery system enables natural, unaltered hydrocortisone to diffuse right into the epidermis. In this way, the anti-inflammatory effect of hydrocortisone is enhanced without resorting to chemical manipulation, making Alphaderm as effective as the fluorinated topical steroids.[1, 2]

Also, Alphaderm contains only natural ingredients in a pleasant, hydrating base, and does *not* include lanolin, parabens or other sensitizing preservatives. It has been shown to be significantly preferred by patients.[2]

References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91**, 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr.med.Res.Opin.* **2**, 474

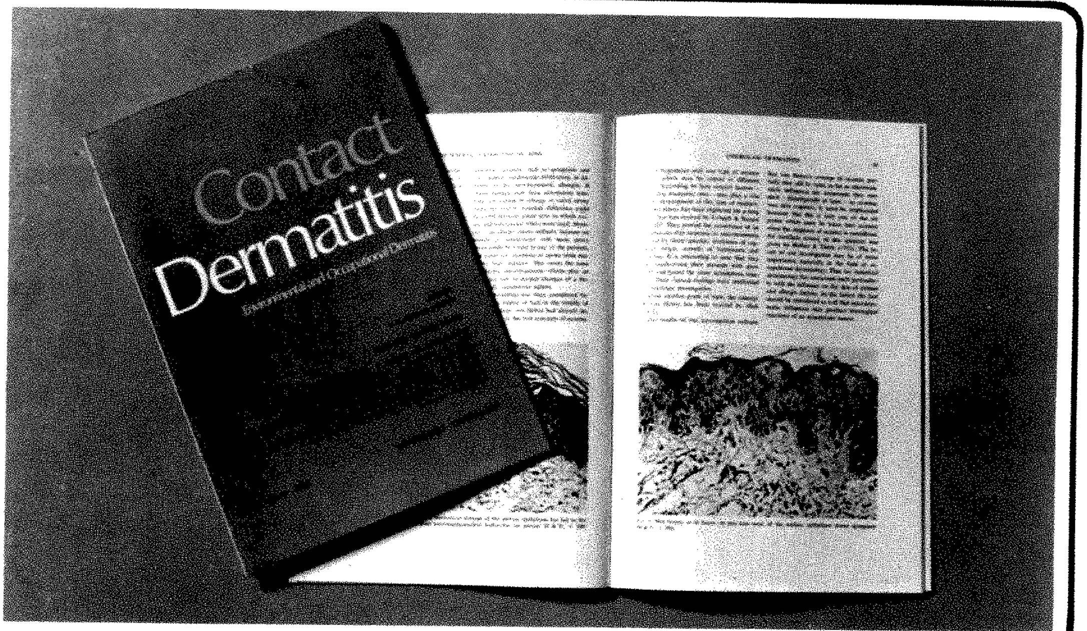
Alphaderm contains 1% hydrocortisone in a special drug-delivery system containing urea: available in 30g tubes and 100g jars.
'Alphaderm' is a trade mark.

Full information on request.
Eaton Laboratories
Regent House,
The Broadway,
Woking, Surrey GU21 5AP

alphaderm

more natural and less hazardous in eczema and dermatitis

Dermatology



Contact Dermatitis

Environmental and Occupational Dermatitis

Editor: C. D.Calnan, London

Contact Dermatitis is designed primarily as a journal for clinicians who are interested in various aspects of environmental dermatitis. This includes both allergic and irritant (toxic) types of contact dermatitis, occupational (industrial) dermatitis and consumers' dermatitis from such products as cosmetics and toiletries.

The journal aims at promoting and maintaining communication between dermatologists, industrial physicians, allergists and clinical immunologists, as well as chemists and research workers involved in industry and the production of consumer goods.

As a special offer to new subscribers to CONTACT DERMATITIS we can offer you volume 1 (1975) FREE as long as the stock lasts so you can have the journal right from the beginning.

MUNKSGAARD · COPENHAGEN

Order Form to be sent in an envelope to:

MUNKSGAARD
35,Nørre Søgade
DK-1370 Copenhagen K.
Denmark

CONTACT DERMATITIS

☐ Please enter my subscription to start with volume 2, 1976. Subscription price D.Kr. 240.00 plus postage D.Kr. 16.00 (US$ 43.50 incl. postage).

☐ Please send me volume 1 FREE (special offer to new subscribers only).

☐ I enclose ________ in payment.

Name: ________

Address: ________

Dermatologica

Offizielles Organ der Schweizerischen Gesellschaft für Dermatologie und Venerologie
Official Organ of the Nederlandse Vereniging van Dermatologen
Official Organ of the Belgian Society for Dermatology and Syphiligraphy

Managing Editor: R. Schuppli (Basel) *Editors: G. Achten* (Bruxelles) *J. Delacrétaz* (Lausanne) *L.H. Jansen* (Utrecht) *A. Kint* (Gent) *C.M. Lapière* (Liège) *J.W. Mali* (Nijmegen) *M.K. Polano* (Leiden) *U.W. Schnyder* (Heidelberg)

Index Vol. 151, No. 5, 1975

Editorial

Jung, E.G. (Mannheim): Sun and Skin

Weirich, E.G. (Basel): Dermatopharmacology of Salicylic Acid. I. Range of Dermatotherapeutic Effects of Salicylic Acid

Arnold, J.; Anthonioz, P., and Marchand, J.P. (Dakar): Depigmenting Action of Corticosteroids. Experimental Study on Guinea Pigs

Abraham, S.; Pandhi, R.K.; Kumar, R.; Mohapatra, L.N., and Bhutani, L.K. (New Delhi): A Study of the Immunological Status of Patients with Dermatophytoses

Rupec, M.; Kint, A.; Himmelmann, G.W. und Brühl, R. (Marburg a.d. Lahn/Gent): Zur Ultrastruktur des soliden Basalioms. Eine morphometrische Untersuchung (Ultrastructure of Solid Basalioma. A Morphometric Study)

Degreef, H.; Debois, J., and Vandepitte, J. (Leuven): *Pseudomonas putrefaciens* as a Cause of Infection on Venous Ulcers

Martin-Pascual, A. and Carcía-Pérez, A. (Salamanca): Botryomycosis

Proceedings · Gesellschaftsberichte · Congrès

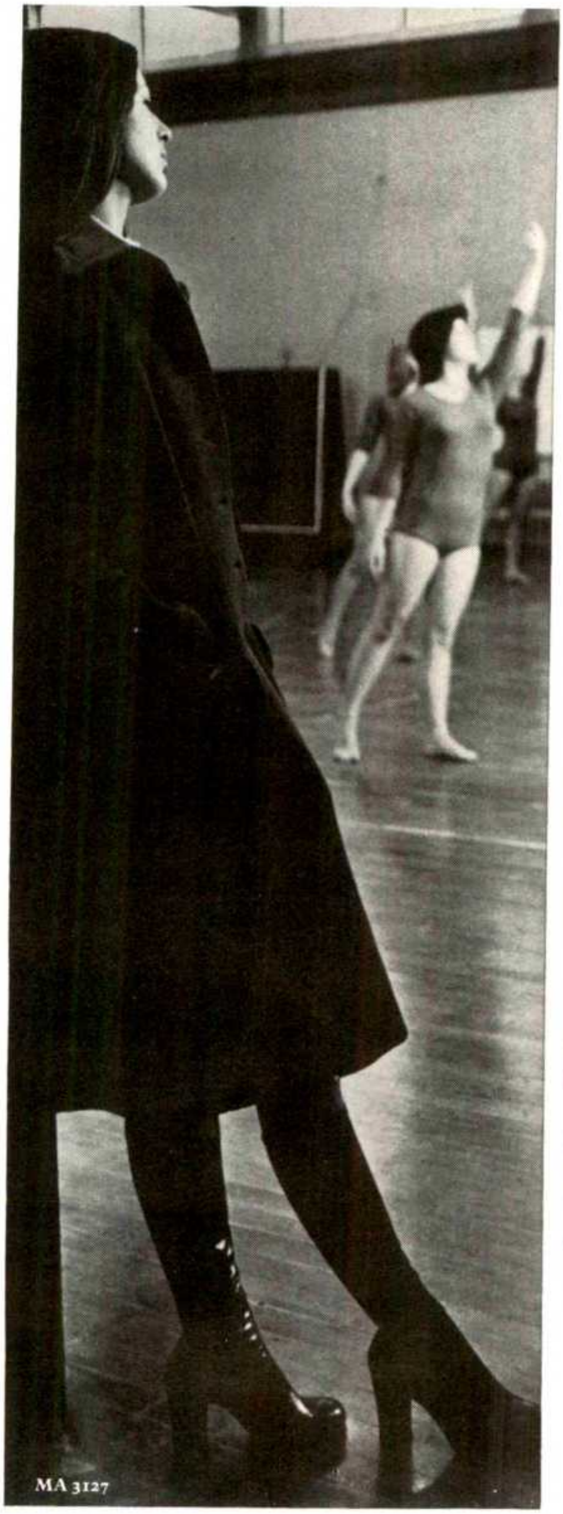
204th Meeting of the Netherlands' Society of Dermatologists, Nijmegen, March 15, 1975

Letters to the Editor
Varia

SI 76001

Subscription data: 'Dermatologica' appears monthly in numbers of approximately 64 pages each. 6 numbers form 1 volume and 2 volumes are issued annually. Subscription price per volume SFr. 110.– / US $ 50.00

S. Karger · Basel · München · Paris · London · New York · Sydney



"Pruritus ani and vulvae is a symptom that can be so annoying, embarrassing, and intractable that sufferers are willing to undertake any treatment to get relief."[1]

'Vallergan'

has so many benefits

antipruritic

'Vallergan' relieves the itching and so stops the scratching. 'Vallergan' reduces the need for any form of topical medication including corticosteroids. 'Vallergan' helps prevent exacerbation and secondary infection.

✻ analgesic

'Vallergan' relieves irritation and pain.

✻ antihistaminic

'Vallergan' is most effective in urticaria and other allergic skin disorders.

✻ sedative

'Vallergan' promotes sleep at night.

✻ well tolerated

'Vallergan' is well tolerated by infants, children and adults, including the elderly.

✻ presentations

You can prescribe 'Vallergan' either as tablets or as syrup, available in two strengths.

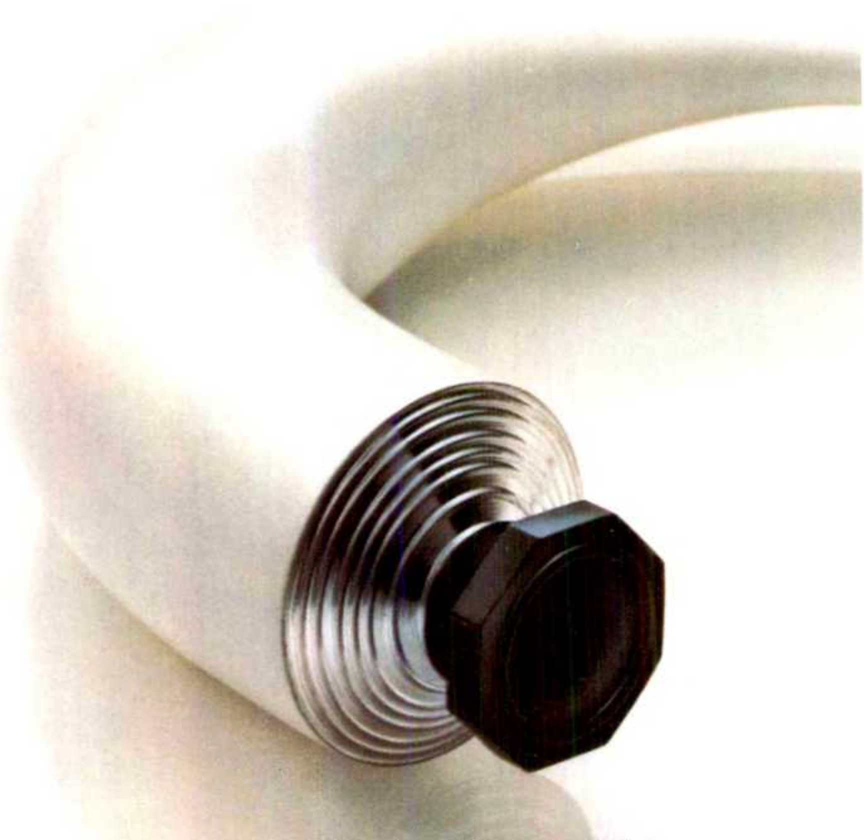
'Vallergan' for itching, scratching, and irritation in all conditions at all sites, at all ages, whatever the cause

Reference 1 Olansky, S.; in *Current Therapy*; edited by H. F. Conn, London: Saunders, 1969, p.646.

Full information is available on request
'Vallergan' is a trade mark of
May & Baker Ltd Dagenham Essex RM10 7XS
for its preparations of trimeprazine tartrate.

M&B May & Baker

A member of the Rhône-Poulenc Group of Companies



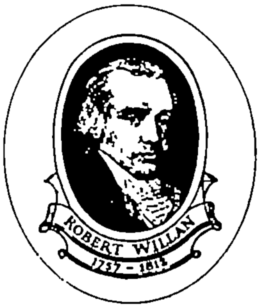
Topical steroid effectiveness is only half the Locoid story

...the other half is the relative freedom from topical side effects which are often encountered with the fluorinated steroids. Locoid offers the same level of success as these potent preparations–without fluorination.

Locoid is a trade mark for hydrocortisone 17-butyrate.
Full information available on request:
Brocades (Great Britain) Ltd.,
Brocades House, West Byfleet, Surrey, KT14 6RA.
Telephone: Byfleet 45536.

locoid

the non-fluorinated way to topical steroid success



British Journal of Dermatology

Volume 95

July – December 1976

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

British Journal of Dermatology

EDITOR
DR R. H. CHAMPION
Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

DR J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London
DR RONALD MARKS, Cardiff
DR P.D.MIER, Nijmegen
DR J.N.S.MITCHELL, Royal Air Force
DR W.C.NOBLE, London
DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS
DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 U.K. (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed in Great Britain at the Alden Press, Oxford

Contents

No. 1: JULY 1976

Clinical and Laboratory Investigations

PAGE

Leukotactic Properties of Soluble Substances in Psoriasis Scale 1
HACHIRO TAGAMI AND SHIGEO OFUJI

Carbohydrate Metabolism in Lichen Planus 9
N. J. LOWE, A. G. CUDWORTH, S. A. CLOUGH AND M. F. BULLEN

Excision Repair of DNA in some Photodermatoses 13
M. ICHIHASHI AND C. A. RAMSAY

A Follow-up Study of Patients with Recurrent Urticaria and Hypersensitivity to Aspirin, Benzoates and Azo Dyes 19
ANN-MARI ROS, LENNART JUHLIN AND GERD MICHAËLSSON

The Role of Divalent Cations in Epidermolysis 25
ROBERT L. DIMOND, KENT L. ERICKSON AND KIRK D. WUEPPER

Necropsy and Ultrastructural Findings in Histiocytic Medullary Reticulosis 35
J. PIÑOL-AGUADÉ, J. FERRANDO, J. M. TOMÁS, C. MIERAS AND J. PEYRI

Leucosis and Skin: Acute Lymphoid Immunoblastic Leukaemia 45
M. G. BERNENGO, A. ZINA AND G. ZINA

Hypersensitivity of Scleroderma Cutaneous Vascular Smooth Muscle to 5-hydroxytryptamine 51
R. K. WINKELMANN, MARC E. GOLDYNE AND RONALD L. LINSCHEID

The Prognosis of Systemic Sclerosis 57
N. R. ROWELL

An Auto-antibody Profile in Alopecia Totalis and Diffuse Alopecia 61
REBECCA E. I. COCHRAN, JOHN THOMSON AND RODERICK N. M. MACSWEEN

Immunological Demonstration of Multiple Esterases in Human Eccrine Sweat 61
W. P. HERRMANN AND J. HABBIG

Pharmacology and Treatment

Septrin in the Treatment of Dermatitis Cruris Pustulosa et Atrophicans 71
WITOLD K. JACYK

The Treatment of Psoriatic Nail Dystrophy with Intradermal Steroid Injections 75
R. D. G. PEACHEY, R. J. PYE AND R. R. M. HARMAN

High Dose Dapsone Toxicity 79
WALTER B. SHELLEY AND MANFRED I. GOLDWEIN

Ciclopirox: a New Topical Pyrodonium Antimycotic Agent. A Double-blind Study in Superficial Dermatomycoses 83
V. N. SEHGAL

Case Reports

Prurigo Nodularis and Gluten Enteropathy 89
A. W. MCKENZIE, D. G. STUBBING AND B. L. ELVY

Sarcoidosis in a Child, Presenting as an Erythroderma with Keratotic Spines and Palmar Pits 93
J. G. L. MORRISON

Brief Communications

Pemphigus-like Syndrome Induced by D-penicillamine 99
S. G. TAN AND N. R. ROWELL

Beta-carotene in the Treatment of Discoid Lupus Erythematosus 100
PETER C. H. NEWBOLD

Systemic Use of a New Retinoid With and Without Local Dithranol Treatment in Generalized Psoriasis 101
C. E. ORFANOS AND U. RUNNE

Correspondence

Is Actinic (Solar) Damage the Provoking Cause of 'Post-inflammatory Elastolysis and Cutis Laxa (PECL)'? 105
JOHN P. O'BRIEN

Causation of Psoriatic Epithelial Hyperproliferation 106
DAVID PASLIN

Book Reviews 106

Review

The Clinical Importance of 'Weathering' in Human Hair 111
A. J. ROOK

News and Notices 113

No. 2: AUGUST 1976

PAGE

Clinical and Laboratory Investigations

Kinetics of *In Vitro* Bovine Keratohyalin Synthesis 115
PETER M. ELIAS, WALTER G. KLINGLER AND PAUL M. MONTAGUE

A Study of the Permeability Barrier in Epidermis and Oral Epithelium using Horseradish Peroxide as a Tracer *In Vitro* 123
C. A. SQUIER AND ROSAMUND M. HOPPS

Study of an Inhibiting Factor of Epidermal Proliferation in Plucked Skin and Various Tumours of Mice 131
M. GUIGON, E. FRINDEL, J. HEWITT AND M. TUBIANA

Sociological Implications of an Epidemiological Study of Eczema in the City of Birmingham 137
N. C. BOWKER, K. W. CROSS, E. A. FAIRBURN AND M. WALL

Complement Component Profiles in Urticaria, Dermatitis Herpetiformis, and Alopecia Areata 145
L. BERRENS, E. JANKOWSKI AND I. JANKOWSKI-BERNTSEN

Pilo-sebaceous Duct Physiology. III. Observations on the Number and Size of Pilo-sebaceous Ducts in Acne Vulgaris 153
W. J. CUNLIFFE, W. D. H. PERERA, P. THACKRAY, M. WILLIAMS, R. A. FORSTER AND S. M. WILLIAMS

Erythropoietic Protoporphyria. Hepatic Implications 157
D. M. MACDONALD AND D. C. NICHOLSON

HL-A Antigens in Patients with Guttate Psoriasis 163
R. C. WILLIAMS, A. W. MCKENZIE, J. H. ROGER AND V. C. JOYSEY

HL-A Antigens in Lichen Planus 169
N. J. LOWE, A. G. CUDWORTH AND J. C. WOODROW

HL-A Antigens and Vitiligo 173
G. RETORNAZ, H. BETUEL, J. P. ORTONNE AND J. THIVOLET

Pharmacology and Treatment

Clinical and Bacteriological Evaluation of a Twice Daily Regimen of Epicillin Capsules for the Treatment of Skin Infections 177
M. EL-ZAWAHRY, ABBAS ABDEL AZIZ, MOHSEN SOLIMAN, S. SHOEIB AND S. S. TADROS

Erythema Multiforme in Children. Response to Treatment with Systemic Corticosteroids 181
JAMES E. RASMUSSEN

Studies on the Effect of Salicylic Acid on Normal Skin 187
M. DAVIES AND R. MARKS

Dithranol (Anthralin) Versus Triacetoxyanthracene. Investigations *In vitro* 193
WOLFGANG P. RAAB

Case Reports

Mudi-chood on the Pinnae 197
P. SUGATHAN

Multiple Pigmented Naevi in an Albino 199
M. HALL, V. EUSEBI AND I. D'ANGELO

Leg Ulcers in a Family with both Beta Thalassaemia and Glucose-6-phosphate Dehydrogenase Deficiency 203
S. GANOR AND T. COHEN

Glaucoma Following the Application of Corticosteroid to the Skin of the Eyelids 207
R. BEVIS CUBEY

Society Proceedings

Dowling Club Proceedings 209
(Chairman: DR R. P. WARIN)

Book Reviews 215

Review

Treatment of Obesity. Appetite Suppressant Drugs and Intestinal By-pass Surgery 219
S. S. BLEEHEN, I. R. EDWARDS AND R. G. CLARK

News and Notices 223

No. 3: SEPTEMBER 1976

Clinical and Laboratory Investigations

PAGE

Epithelioma Adenoides Cysticum: Genetic Update 225
DAVID E. ANDERSON AND JAMES B. HOWELL

Transformation of Epithelioma Adenoides Cysticum into Multiple Rodent Ulcers: Fact or Fallacy. A Historical Vignette 233
J. B. HOWELL AND DAVID E. ANDERSON

Histopathological Spectrum of Erythema Multiforme 243
T. R. BEDI AND HERMANN PINKUS

Histology Simulating Reticulosis in Secondary Syphilis 251
REBECCA E. I. COCHRAN, JOHN THOMSON, K. A. FLEMING AND ALEXANDRA M. M. STRONG

Changes in Transepidermal Water Loss and the Composition of Epidermal Lecithin after Applications of Pure Fatty Acid Triglycerides to the Skin of Essential Fatty Acid-deficient Rats 255
P. J. HARTOP AND C. PROTTEY

The Diffusion of Nandrolone through Hydrated Human Cadaver Skin 265
M. I. FOREMAN AND I. KELLY

Lysosomal Hydrolases of the Epidermis. 6. Changes in Disease 271
P. D. MIER AND JOSÉ J. M. A. VAN DEN HURK

The Fate of Staphylococcal Exfoliatin in Newborn and Adult Mice 275
PETER FRITSCH, PETER ELIAS AND JANOS VARGA

Hypersensitivity to Bacteria in Eczema. II. Titre and Immunoglobulin Class of Antibodies to Staphylococci and Micrococci 285
W. E. PARISH, ESTHER WELBOURN AND R. H. CHAMPION

Immunological Aspects of Psoriasis. II. Dissociated Impairment of Thymus-Dependent Lymphocytes 295
J. J. GUILHOU, J. MEYNADIER, J. CLOT, E. CHARMASSON, M. DARDENNE AND J. BROCHIER

Alternative Pathway Complement Activation: A Possible Mechanism Inducing Skin Lesions in Benign Gonococcal Sepsis 303
REINER SCHERER AND OTTO BRAUN-FALCO

The Effect of Long Term Tetracycline Treatment for Acne Vulgaris on the Occurrence of R Factors in the Intestinal Flora of Man 311
M. V. VALTONEN, V. V. VALTONEN, O. P. SALO AND P. H. MÄKELÄ

Pharmacology and Treatment

A Double-blind Trial of Clomocycline in the Treatment of Persistent Palmoplantar Pustulosis 317
JOANNA M. WARD, MARGARET F. CORBETT AND M. J. HANNA

Co-operative Double-blind Trial of an Antibiotic/Corticoid Combination in Impetiginized Atopic Dermatitis 323
GERALD N. WACHS AND HOWARD I. MAIBACH

Brief Communications

Corticotrophin and Vitiligo (Preliminary Observations) 329
B. B. GOKHALE AND TARA B. GOKHALE

Muscle Herniation of the Lower Legs 329
JULIAN VERBOV

Canadian–Irish Dermatological Meeting 330

Book Reviews 332

Review

Adverse Reaction to the β-adrenergic Blocking Drugs 335
W. D. BRIGDEN AND J. ALMEYDA

News and Notices 339

No. 4: OCTOBER 1976

Clinical and Laboratory Investigations

PAGE

Practical Applications of the Quantimet 720, an Image Analysing Computer, in the Field of Investigative Dermatology ... 341
W. PICTON, H. DEVITT AND M. A. FORGIE

The Lines of Blaschko: a Review and Reconsideration. Observations of the Cause of Certain Unusual Linear Conditions of the Skin ... 349
ROBERT JACKSON

The Effect of Collagenase on Surface Markers of Human Peripheral Lymphocytes ... 361
TULIP TAN

Infantile Eczema. A Long-term Follow-up Study ... 365
KEITH MUSGROVE AND JOHN K. MORGAN

Psychological Profile of Patients with Atopic Dermatitis ... 373
HANA F. AL-AHMAR AND AMAL K. KURBAN

Hypersensitivity to Bacteria in Eczema. III. Arthus-like Responses to Bacterial Antigens Without Specific Antibody ... 379
ESTHER WELBOURN, R. H. CHAMPION AND W. E. PARISH

Pigmented Lesions in Newborn Infants ... 389
ROBERT G. WALTON, ALVIN H. JACOBS AND ALVIN J. COX

The Effects of Photosensitizers and Ultraviolet Irradiation on the Biosynthesis and Metabolism of Prostaglandins ... 397
JONATHAN T. LORD, VINCENT A. ZIBOH, JOSEPH POITIER, GERALD LEGGET AND NEAL S. PENNEYS

Subcutaneous Fat Necrosis of the Newborn. Light and Electron Microscopic Studies ... 407
TAKUO TSUJI

Metal Corrosion by Chloride in Sweat. The Problem of 'Rusters' in Industry ... 417
J. L. BURTON, R. J. PYE AND D. B. BROOKES

Pharmacology and Treatment

Proprietary Hydrocortisone Creams. Vasoconstrictor Activities and Bio-availabilities of Six Preparations 423
B. W. BARRY AND R. WOODFORD

Effect of 1% Cyproterone Acetate in Cetomacrogol Cream BPC (Formula A) on Sebum Excretion Rate in Patients with Acne ... 427
R. J. PYE, J. L. BURTON AND J. I. HARRIS

Case Reports

Disseminated Superficial Actinic Porokeratosis. Analysis of an Affected Family ... 429
DONALD J. PIROZZI AND ALBERT ROSENTHAL

Unilateral Epidermal Naevus Resembling Acanthosis Nigricans ... 433
HELEN OLLENDORFF CURTH

Reticulate Acropigmentation of Kitamura ... 437
W. A. D. GRIFFITHS

Pemphigus Induced by Rifampicin ... 445
R. W. GANGE, E. L. RHODES, C. O. EDWARDS AND M. E. A. POWELL

Comment

Characteristics and Clinical Importance of Killer (K)-cells ... 449
DIETRICH KRAFT

Ph.d Theses and Book Reviews ... 453

News and Notices ... 457

No. 5: NOVEMBER 1976

Clinical and Laboratory Investigations

PAGE

The Sunburn Cell in Mouse Skin: Preliminary Quantitative Studies on its Production ... 459
A. WOODCOCK AND I. A. MAGNUS

Thymus-dependent (T) Lymphocyte Deficiency in Progressive Systemic Sclerosis ... 469
P. HUGHES, SHIRLEY HOLT, N. R. ROWELL AND JANIS DODD

Macrophage Inhibitor Factor (MIF) in Cutaneous Lymphoproliferative Diseases 475
PABLO UMBERT, RICHARD W. BELCHER AND R. K. WINKELMANN
Lymphokines (MIF) in the Serum of Patients with Sarcoidosis and Cutaneous Granuloma Annulare 481
PABLO UMBERT, RICHARD W. BELCHER AND R. K. WINKELMANN
Granuloma Annulare: Direct Immunofluorescence Study 487
PABLO UMBERT AND R. K. WINKELMANN
Hypersensitivity to Bacteria in Eczema. IV. Cytotoxic Effect of Antibacterial Antibody on Skin Cells Acquiring Bacterial Antigens 493
W. E. PARISH, ESTHER WELBOURN AND R. H. CHAMPION
The Ultrastructure of the Human Epidermis Following Ultracentrifugation 507
G. H. FINDLAY AND N. V. D. W. LIEBENBERG
The Epidermis in Thyroid Disease 513
P. J. A. HOLT, J. LAZARUS AND R. MARKS
Plasma Volume, Intravascular Albumin and its Transcapillary Escape Rate in Patients with Extensive Skin Disease 519
HANS-HENRIK PARVING, ANNE-MARIE WORM AND NIELS ROSSING
Physical Training and Connective Tissues in Young Mice. Biochemistry of Skin 525
A. KIISKINEN AND E. HEIKKINEN
Localized Pemphigoid 531
JOHN R. PERSON, ROY S. ROGERS III AND HAROLD O. PERRY
Bullous Pemphigoid and Psoriasis: Does Subclinical Bullous Pemphigoid Exist? 535
JOHN R. PERSON AND ROYS S. ROGERS III
Bullous Dermatosis of Chronic Renal Failure 541
KALMAN KECZKES AND MALCOLM FARR

Case Reports

Caustic Ulcers Caused by Cement 547
MATTI HANNUKSELA, RAIMO SUHONEN AND JAAKKO KARVONEN
Metastatic Crohn's Disease 551
D. I. MCCALLUM AND W. M. GRAY
Paroxysmal Itching in Multiple Sclerosis 555
P. O. OSTERMAN
Generalized Warts and Immune Deficiency 559
T. M. S. REID, N. G. FRASER AND I. R. KERNOHAN

Brief Communications

Improved Technique for Epidermal Cell Cycle Analysis 565
F. W. BAUER AND R. M. DE GROOD
Effect of Hydrocortisone Butyrate on Collagen Synthesis 566
HEIKKI SAARNI AND VÄINÖ K. HOPSU-HAVU
PUVA-induced Bullous Pemphigoid 568
KRISTIAN THOMSEN AND HENNING SCHMIDT

Theses and Book Reviews 571

News and Notices 575

No. 6: DECEMBER 1976

Clinical and Laboratory Investigations PAGE

The Cell Cycle in Psoriasis: a Reappraisal 577
SEYMOUR GELFANT
Localization and Activity of Tissue Bound Cyclic Nucleotide Phosphodiesterase in Normal and Lack of Changes in Psoriatic Human Skin 591
G. MAHRLE AND C. E. ORFANOS
Periodic Acid-Schiff (PAS) Staining for Glycogen in Clinically Normal Psoriatic and Non-psoriatic Skin 599
LESLIE STANKLER AND FREDERICK WALKER
Bacterial Flora in Psoriasis 603
RAZA ALY, HOWARD I. MAIBACH AND ADRIAN MANDEL

Effects of Occlusion upon Population Dynamics of Skin Bacteria 607
DAVID J. BIBEL, DAVID J. LOVELL AND ROKO J. SMILJANIC
Airborne Contact Dermatitis from Compositae Oleoresins Simulating Photodermatitis 613
NIELS HJORTH, JYTTE ROED-PETERSEN AND KRISTIAN THOMSEN
Changes in Dry Weight and Projected Area of Human Epidermal Cells Undergoing Keratinization as Determined by Scanning Interference Microscopy 621
H. J. YARDLEY AND D. J. GOLDSTEIN
A Lysosomal Storage Disorder of the Epidermis Characterized by a Deficiency of α-Mannosidase and an Accumulation of Mannose-rich Materials 627
J. W. H. MALI, A. M. G. BERGERS, JOSÉ J. M. A. VAN DEN HURK, P. D. MIER AND W. J. B. M. VAN DE STAAK
Histological and Direct Immunofluorescence Study of Cutaneous Hyperreactivity in Behçet's Disease 631
S. HAIM, J. D. SOBEL, R. FRIEDMAN-BIRNBAUM AND C. LICHTIG
Familial Behçet's Syndrome 637
S. K. GOOLAMALI, J. S. COMAISH, F. HASSANYEH AND A. STEPHENS

Pharmacology and Treatment

Relapse Rate of Psoriasis Worsened by Adding Steroids to a Dithranol Regime 643
R. H. SEVILLE

Case Reports

Epidermal Naevus Syndrome 647
M. K. ATTIA AND A. M. ABDEL-AZIZ
Atrophie Blanche Lesions Closely Resembling Malignant Atrophic Papulosis (Degos' Disease) in Systemic Lupus Erythematosus 649
MARTIN M. BLACK AND PETER M. HUDSON
Adverse Skin Reaction from Vitamin K1 653
H. M. BARNES AND I. SARKANY
Congenital Triangular Alopecia 657
RAJ KUBBA AND ARTHUR ROOK
Eosinophilic Pustular Folliculitis. Report of a European Case 661
ROLF HOLST

Comment Article

Langerhans Cells: Involvement in the Pathogenesis of Mycosis Fungoides 665
G. ROWDEN AND M. G. LEWIS

Brief Communications

Fibrinolytic Agents in the Treatment of Necrobiosis Lipoidica 673
E. L. RHODES
Sodium Potassium and Phosphorus Content of Normal and Psoriatic Skin 674
G. H. HAJINI, S. T. HUSSAIN AND S. N. A. SHAH
First Demonstration of Cathepsin A in Epidermis 675
G. VOLDEN, JOSÉ J. M. A. VAN DEN HURK, P. D. MIER AND H. ROELFZEMA

Obituaries

Dr G. B. Dowling 677
Dr J. Savatard, Dr W. B. Reed, Dr M. Farb and Dr W. W. Gunther 681

Correspondence 682

Book Reviews 682

News and Notices 686

Index to Volume 95 691

British Journal of Dermatology (1976) 95, 1.

Clinical and Laboratory Investigations

Leukotactic properties of soluble substances in psoriasis scale

HACHIRO TAGAMI AND SHIGEO OFUJI

Department of Dermatology, Kyoto University, Faculty of Medicine, Sakyo-ku, Kyoto, Japan 606

Accepted for publication 17 November 1975

SUMMARY

In an attempt to elucidate the mechanisms underlying the production of transepidermal migration of leukocytes toward the stratum corneum in psoriatic lesions, the chemotactic properties of soluble substances in psoriasis scales were examined by a modified Boyden's chamber.

All crude extracts of horny tissues studied, i.e. callus, scales of exfoliative dermatitis and of psoriasis vulgaris, showed chemotactic activity for human peripheral blood leukocytes. But only the chemotactic activity of the psoriasis scale extract was highly potent. This was greatly reduced after dialysis. Fresh serum was not required to manifest the chemotactic activity.

By Sephadex G-200 chromatography, the scale extracts from psoriasis vulgaris and pustular psoriasis had potent activity eluted near the cytochrome C marker. The same fractions of other horny tissue extracts, bacterial filtrate prepared from cultured psoriasis scale fragments, and serum did not show such potent activity.

On the basis of analysis by gel filtration and recent findings of immunopathological studies, a postulate was made that a complement derived chemotactic factor, possibly a C5 cleavage product, developed as a result of antigen–antibody reaction in the stratum corneum of psoriatic lesions.

One of the most characteristic histological features in psoriatic lesions is the 'squirting papillae', i.e. the rhythmical discharge of leukocytes from the tips of engorged capillaries in the dermal papillae through breaks in the basement membrane, which in turn lead to formation of Munro's micro-abscesses in the stratum corneum (Pinkus & Mehregan, 1966). The migrating leukocytes are lymphocytic cells and polymorphonuclear leukocytes (PMN) in ordinary lesions (Soltani & Van Scott, 1972), but the latter predominate in the acute exudative and pustular forms. Langhof & Müller (1966) demonstrated by a slide and coverslip preparation, and a photomicrographic trace recording technique, that ultrafiltered homogenates of psoriasis scales exhibited chemotactic activity for human peripheral blood leukocytes in plasma. No information is available, however, as to whether or not the leukotactic properties of the horny tissues are specific only for psoriasis scale or if plasma is required to manifest the leukotactic effect.

The study of leukocyte chemotaxis has been facilitated by Boyden's (1962) micropore filter technique, which enables measurement of chemo-attractants more quantitatively and analytically than previous *in vitro* methods. Thus, a distinction has been made between substances which exert a direct chemotactic effect on leukocytes (cytotaxins) and those which act indirectly, producing a chemotactic effect only in the presence of plasma or serum (cytotaxigens) (Keller & Sorkin, 1967).

Using a modified Boyden's micropore filter technique the present study was designed to measure analytically the chemotactic properties of psoriasis scale compared with those of other horny tissues, skin bacteria filtrates and plasma components.

MATERIALS AND METHODS

Extracts of horny tissues

Water-soluble substances were extracted from the following horny tissues: (1) psoriatic scales collected from non-treated lesions of two patients with psoriasis vulgaris, a man aged 46 years and a woman aged 68 years, and exfoliated scales from the bed sheets of an 11-year-old girl with pustular psoriasis at the time of exacerbation; (2) exfoliated scales from the bed sheets of two patients with exfoliative dermatitis of non-psoriatic origin, a 68-year-old man and a 46-year-old woman; and (3) pooled samples of callus and clavus obtained from healthy volunteers and patients with psoriasis vulgaris. Although the chemical composition of normal stratum corneum differs slightly from that of callus (Fisher, 1965; Jacobi, 1971), in a preliminary study it was noted that the callus extract and an extract of epidermal sheets prepared from hypertrophic scars obtained at surgery showed a similar chemotactic behaviour. Thus callus was used in order to obtain sufficient amounts of orthokeratotic horny layers instead of normal horny layers.

The water-soluble components were extracted in physiological saline (later, 0·15 M phosphate buffered saline (pH 7·4) was also used, with similar results). All materials were dried in a dessicator before use, weighed, washed in saline containing 0·1% streptomycin, cut into tiny pieces, homogenized in a glass homogenizer and suspended in saline at 200 times the original dry weight (1:200 extracts). For preparations to be separated by gel chromatography, homogenized materials were suspended in saline of 20 times the original dry weight (1:20 extracts). The homogenates were shaken at 4°C for 7 days and centrifuged at 12,000 rev/min for 15 min. The supernatants were sterilized by Millipore filtration, streptomycin was added to make a final concentration of 100 μg/ml and then chemotactic assays were carried out. To obtain extracts free of low molecular weight substances, these solutes were further dialyzed against repeated changes of saline at 4°C. Since the extract prepared from callus loses most of its soluble components with dialysis, highly concentrated solutions were prepared after lyophilization. Protein was estimated by the method of Lowry *et al.* (1951).

Filtrates from bacterial cultures

A tiny fragment of psoriatic scales freshly obtained from non-treated psoriatic lesions was cultured in Hank's solution with 0·5% bovine serum albumin (pH 7·4; Hank-BSA) for 2–6 days at 37°C and supernatants were sterilized by Millipore filtration.

Cell preparations

Leukocyte suspensions were prepared from peripheral blood of healthy volunteers, patients with psoriasis and those with various dermatoses other than psoriasis. None of them was receiving systemic steroids or cytostatic agents. Seven ml of blood were drawn in heparin (3U/ml) and after addition of 6% dextran (Nakarai Chemical Co.) in saline was allowed to sediment at a room temperature for 30 min. The white blood cell rich supernate was spun for 10 min at 750 rev/min and the white blood cell pellet was washed 3 times with Hank's solution before suspension in Hank-BSA (pH 7·4) to a final concentration of 2×10^6 cells/ml. Thus prepared leukocyte suspensions contained 50–70% polymorphonuclear leukocytes (PMNs).

Chemotactic assay

The modified Boyden's chamber study resembled that described by Hurley (1963). The bottom compartment was a glass tube with an inside diameter of 1·4 cm and a length of 1·8 cm with a plastic ring 2 mm in thickness fitted to the bottom. The top compartment, which contained the cell suspension, was a transected 2·5 ml disposable plastic syringe (Jintan Pharmaceutical Co.) of 2·5 cm length and 0·9 cm inside diameter. A Millipore filter of 3 μm mean pore diameter was cemented to one end of the tube to form the cell suspension compartment. In testing chemotaxis of leukocytes, 0·1 ml of a given test solution was placed on the bottom compartment with the remaining 0·4 ml of Hank-BSA. The test solutions were run in duplicate. The cell suspensions (0·5 ml containing 10^6 cells) were placed in the upper compartment. The chambers were incubated at 37°C for 3 h. The Millipore filter was fixed in methanol, stained with haematoxylin, cleared in xylene and mounted on a glass slide with the test chamber-side uppermost. For each filter, 10 random fields were counted at a magnification of ×400 and the number of leukocytes migrating completely through to the bottom surface of the filter was recorded. Under the present experimental conditions (Millipore filter of 3 μm pore size and incubation time 3 h), most of the leukocytes migrating completely through to the bottom surface consisted of PMNs. Chemotactic activity was expressed as the average number of cells per high power field.

Chromatography

The chemotactic activity in horny tissue extracts was characterized by gel filtration on Sephadex G-200 (2·5 × 50 cm). Four ml of a given material was added to the column and physiological saline was used as the eluent. Blue dextran, bovine serum albumin (BSA), and cytochrome C were used as molecular markers. The absorbancy of each fraction (6 ml) collected was determined at 280 nm. For measurement of chemotactic activity, 0·5 ml of each fraction was placed to the test compartment without Hank-BSA.

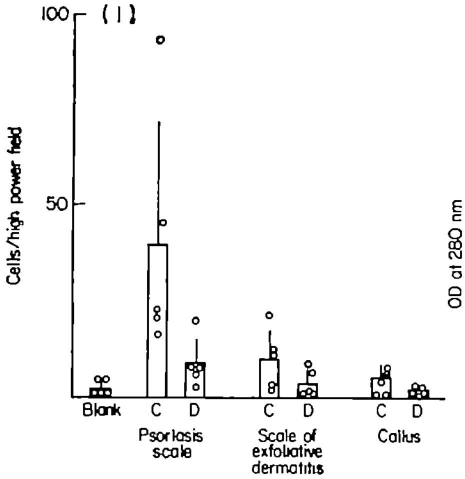
RESULTS

Background level of migration (random filter migration)

The number of leukocytes migrating completely through the filter varied from one subject to the next, ranging from 1 to 19 per high power field. The influence of age and sex of the subjects was not apparent. The mean number of leukocytes ± s.d. from thirty non-psoriatic individuals (mean age 36·9 years) was 5·4±3·2 per field: among them three subjects were healthy volunteers; fourteen had eczematous dermatitis; four superficial fungus infections; three pityriasis rosea; and one each had folliculitis, acne vulgaris, alopecia areata, recurrent aphthosis, vitiligo and lichen planus. The random filter migration values for the subjects with psoriasis vulgaris were generally in the upper range compared to those for non-psoriatic subjects. When the data for seventeen psoriatic patients (mean age 36·1 years) were analyzed as a group and the mean number ± s.d., 7·5±5·9, compared to that of thirty non-psoriatics, the mean value was found to be higher but not significantly ($P < 0{\cdot}1$). Leukocytes from two patients with pustular psoriasis, a 46-year-old male and an 11-year-old girl, also did not show any particular difference in random filter migration; their values were 2 and 4 cells per high power field respectively.

Chemotactic properties of psoriasis scale extract

In Fig. 1 the comparison of chemotactic activity of various horny tissue extracts prepared in the same way (1:200 extracts) is shown, which was carried out using leukocytes taken from five subjects. The psoriasis scale extract (pH 5·4) showed very strong chemotactic activity, while the extracts of scales



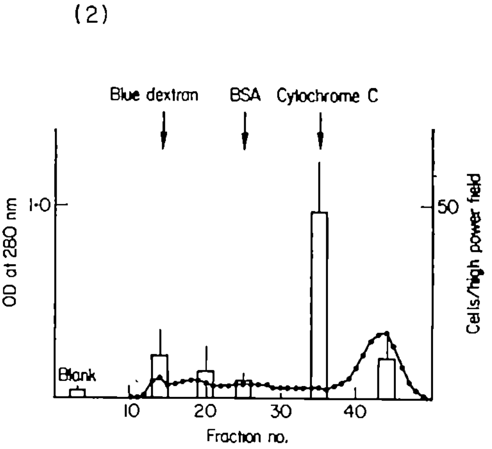


FIGURE 1. Comparison of leukotactic activity of extracts from various horny tissues; crude and dialyzed preparations. Assayed with leukocytes obtained from five subjects. C = crude extract; D = dialyzed extract.

FIGURE 2. Characterization, by Sephadex G-200 chromatography, of chemotactic activity in crude psoriasis scale extract. Chemotactic activity was measured with leukocytes prepared from four subjects. Columns, cells/high power field.

TABLE 1. Comparison of chemotactic response in leukocytes from non-psoriatic and psoriatic subjects

	Chemotactic activity (cells/high power field) material tested	
Source of peripheral blood leukocytes	Blank	1:200 Crude psoriatic scale extract
Non-psoriatics		
1	6	15
2	4	13
3	3	10
4	6	48
5	10	30
Average	5·8±2·7	23·2±15·9
Psoriatics		
1	13	30
2	10	76
3	9	44
4	9	20
5	5	35
Average	9·2±2·7	41·0±18·9

from exfoliative dermatitis and of callus were only weakly chemotactic and not at a statistically significant level compared to background levels of migration (blank). No specific deviation in chemotactic response to the psoriasis scale extract was observed with leukocytes from psoriatic subjects as compared to non-psoriatic leukocytes, although the former showed more active migration (Table 1).

The activity was heat stable at 56°C for 30 min and was retained after neutralization of the pH by dialysis against PBS (pH 7·4) (Table 2). The chemotactic activity was likewise retained after neutralization with 1 N NaOH (data not shown), in contrast to the observation made by Langhof & Müller (1966) that there was loss of leukotaxy with such preparations. Upon dialysis the chemotactic potency was greatly reduced but still retained in this preparation (Fig. 1).

The Sephadex G-200 elution profile revealed two major components in regard to uv absorbance; the first several low peaks of non-dialyzable fractions and the second high symmetrical monophasic peak of low molecular weight fractions (Fig. 2). Moderate chemotactic activity was observed in a multiphasic pattern. However, prominent chemotactic activity was observed in the fractions 30–38, eluting near the cytochrome C marker. Judging from the low uv absorbance, it was apparent that highly chemotactic substances were contained in these fractions. The soluble components of scales from pustular psoriasis also showed a similar elution profile. In regard to chemotactic activity the prominent activity was also noted at the same fractions as those of the scale extract from psoriasis vulgaris (Table 3).

TABLE 2. Leukotactic properties of psoriasis scale extract after heating for 30 min at 56°C or after dialysis against phosphate buffered saline (pH 7·4)

Subject	Blank	Psoriasis scale extract heated for 30 min at 56°C	Psoriasis scale extract dialyzed against PBS (pH 7·4)
1	3	12	—
2	3	17	—
3	2	33	—
4	5	—	9
5	3	—	9
6	12	—	19

TABLE 3. Comparison of chemotactic activity of fraction eluting near cytochrome C marker (fraction no. 35) from various horny tissue extracts (1:20 extracts), culture filtrate of psoriasis scale, and serum from a psoriatic individual

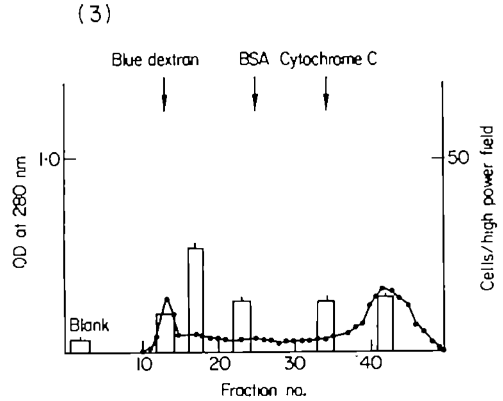
Tissue	OD at 280 nm	Cells/high power field		
		Blank	Fraction no. 35	(No. assay)
Psoriasis scale	0·06	2·5±0·6	48·3±12·3*	(4)
Scales from pustular psoriasis	0·07	4·0±6·7	104·9±46·4†	(4)
Scales from exfoliative dermatitis	0·06	3·5±0·7	13·5± 0·7*	(2)
Callus	0·02	3·3±2·2	4·5± 3·7	(4)
Psoriasis bacterial filtrate	0·01	3·0±0·0	4·0± 2·0	(2)
Serum from psoriatic individual	0·02	2·0±1·0	4·6± 1·5	(3)

* Probability <0·01; † Probability <0·05.

Chemotactic properties of other horny layer extracts

Although the chemotactic activity of scale extract from exfoliative dermatitis was much lower than that of the psoriasis scale extract, this preparation also partially retained its activity after dialysis, which was heat stable at 56°C for 30 min. The Sephadex G-200 elution profile showed a very high peak in the void of the high molecular weight fractions (Fig. 3). Moderate chemotactic activity was noted in high molecular weight fractions. However, the chemotactic activity located near the cytochrome C marker was not so great as that noted with the psoriasis scale extract (Table 3).

In contrast to the above mentioned scale extracts, the weak chemotactic activity was virtually lost from the callus extract upon dialysis. Even the concentrated solution of non-dialyzable components (protein 1·2 mg/ml) was chemotactically inactive (Table 4). These observations are quite obvious from the Sephadex G-200 elution profile; no prominent peaks of high molecular weight were present and there was only a high, monophasic peak of low molecular weight, which was associated with weak chemotactic activity as in the scale extracts. The extract prepared from callus obtained from a psoriatic individual showed the same chemotactic properties.



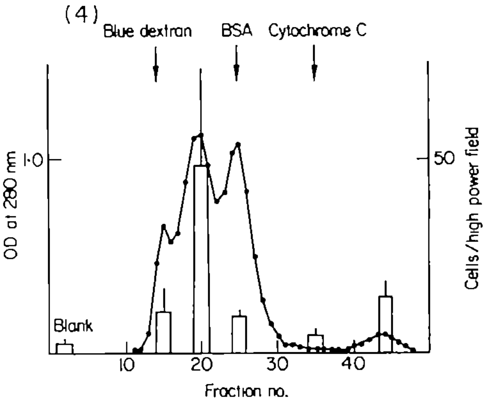


FIGURE 3. Characterization of chemotactic activity in crude scale extract from exfoliative dermatitis, by Sephadex-G 200. Chemotactic assay was carried out with leukocyte preparations obtained from two subjects. Columns, cells/high power field.

FIGURE 4. Chemotactic ability of fractions of serum obtained from a 50-year-old woman with psoriasis vulgaris prepared by Sephadex G-200 chromatography. Chemotactic assay was performed with leukocyte preparations obtained from three subjects. Columns, cells/high power field.

TABLE 4. Leukotactic activity of the concentrated solution of non-dialyzable components of callus extract (protein 1·2 mg/ml)

Subject	Blank	Concentrated dialyzed callus extract
1	6	3
2	5	4
3	5	5
4	3	3
5	2	3
Average	4·2±1·6	3·6±0·9

Chemotactic properties of bacterial filtrates prepared from flora of psoriasis scale

Staphylococcus aureus, *staphylococcus epidermidis* and *Bacillus subtilis* were grown on different occasions from psoriasis scale fragments cultured in Hanks-BSA. At first the growth was rather slow but after 4 days these preparations became turbid and malodorous. Culture filtrates were found to be chemotactically inactive until 4 days, when even that of *Bacillus subtilis* exhibited strong chemotactic activity. The Sephadex G-200 elution profile of the 6-day-culture filtrate of psoriasis scales showed weak chemotactic activity in fractions containing BSA and moderate activity in low molecular weight fractions. The latter was lost totally on dialysis.

Chemotactic properties of serum

Sephadex G-200 chromatography of serum, obtained from either a healthy subject, or a patient with psoriasis vulgaris, or one with pustular psoriasis, showed that both high molecular weight and low molecular weight fractions contained chemotactically active substances (Fig. 4). They were heat stable at 56°C for 30 min. However, the chemotactic activity located near the cytochrome C marker was not demonstrable in non-treated serum.

DISCUSSION

Chemotactic mediators in the stratum corneum or in the upper portion of the epidermis of psoriatic lesions should be demonstrable in view of the fact that the migration and accumulation of leukocytes are observed histologically in the subcorneal portion of the psoriatic epidermis, particularly in the acute and pustular forms (Pinkus & Mehregan, 1966). By means of a slide-coverslip method Langhof & Müller (1966) found that homogenates of psoriasis scale were leukotactic in plasma. In the present study we utilized a modified and simplified Boyden's micropore filter technique. This enabled us to perform chemotactic assays more quantitatively and analytically than the method used by them, although the method leaves still much room for improvement (Keller, Hess, & Cottier, 1975). Our results showed that the psoriasis scale extract exhibits highly potent chemotactic activity as compared to other extracts from various kinds of horny tissues which are also chemotactically active, but only weakly. Serum is not required to manifest the chemotactic effect, i.e. they contain cytotaxins. Langhof & Müller (1966) found that the homogenates of psoriasis scales became chemotactically inert after neutralization of the acidity with 1 N NaOH. However, this was not confirmed in the present study (even those extracts prepared in PBS (pH 7·4) showed comparable results). Leukocytes from psoriatic individuals did not show any qualitative difference in response to the psoriasis scale extract as compared to those from non-psoriatic persons.

Saline extraction of various horny tissues removes abundant dialyzable low molecular weight compounds, which are moderately chemotactically active in all the crude extracts, plus small amounts of proteinous material. There are several possibilities regarding the origin of the cytotaxins contained in the low molecular weight components, i.e. bacterial cytotaxins, metabolic products of the skin, or components of plasma or tissue fluid diffusing through the epidermis. Among them bacterial cytotaxins are most likely to contaminate the horny layers as exogenous chemotactic factors of low molecular weight (Ward, Lepow & Newman, 1968). The present study reveals that low molecular weight cytotaxins are formed in culture filtrates of skin microflora isolated from psoriatic lesions; profuse proliferation of microflora may take place in psoriatic scales (Marples, Heaton & Kligman, 1973).

Most chemotactic activity of the psoriasis scale extracts elute near the cytochrome C marker (molecular weight, 12,000), even though the amount is rather small. Although chemotactic activity of this eluate is likewise noted in the scale extract from exfoliative dermatitis, it is not as potent as in the scale extracts from psoriasis vulgaris and pustular psoriasis (Table 3). We consider that it contributes to the strong chemotactic potency of the crude psoriatic scale extract. On the basis of this strong chemotactic activity and specificity to psoriasis scale, it is reasonable to assume that this potent cytotaxin plays an important role in leukocyte migration in psoriatic lesions. Of the known endogenous chemotactic factors, the active substance contained in fractions eluting near cytochrome C marker is likely to be a cleavage product of C5 based on analysis by gel filtration (Ward & Newman, 1969). Krogh & Tönder (1973) reported the detection of immunoglobulins and C3 in saline extracts of scales from patients with psoriasis. Recently, *in vivo* fixed IgG and complement have been demonstrated in the intercellular areas of stratum corneum, the site of localization of the stratum corneum antigen, of psoriatic lesions (Beutner *et al.*, 1975; Jablonska *et al.*, 1975). In addition, the presence of various immunoglobulins has been observed in the nuclei of parakeratotic cells of psoriatic scales (Cormane, R.H., Hunyadi, J. & Hamerlinck, F; personal communication). Taking these observations into consideration, it is of interest to speculate whether the potent cytotaxin in the psoriatic scales is a complement-derived chemotactic factor released as a result of the antigen–antibody reaction.

ACKNOWLEDGMENTS

We thank Drs T.Imai, A.Ogino, M.Hiramoto and T.Horio for providing horny tissues and M.Ohara for assistance with the manuscript. This work is supported in part by grant-777182 from the Ministry of Education, Japan and in part by a grant from Fujiwara Memorial Foundation.

REFERENCES

Beutner, E.H., Jablonska, S., Jarzabek-Chorzelska, M., Maciejowska, E., Rzesa, G. & Chorzelski, T.P. (1975) Studies in immunodermatology. VI. IF studies of auto-antibodies to the stratum corneum and of *in vivo* fixed IgG in stratum corneum of psoriatic scales. *International Archives of Allergy and applied Immunology*, **48**, 301.

Boyden, S. (1962) The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. *Journal of Experimental Medicine*, **115**, 453.

Fisher, J.P. (1965) Soluble substances of human stratum corneum. I. Immunochemical and immunological study. *Journal of Investigative Dermatology*, **44**, 43.

Hurley, J.V. (1963) Incubation of serum with tissue extracts as a cause of chemotaxis of granulocytes. *Nature*, **158**, 1212.

Jablonska, S., Chorzelski, T.P., Jarzabek-Chorzelska, M. & Beutner, E.H. (1975) Studies in immunodermatology. VII. Four-compartment system studies of IgG in stratum corneum and of stratum corneum antigen in biopsies of psoriasis and control dermatoses. *International Archives of Allergy and applied Immunology*, **48**, 324.

Jacobi, O. (1971) Die Inhaltsstoffe des normalen Stratum corneum und Callus der menschlicher Haut. III. Milch-säure, Kreatin, Kreatinin, Harnstoff und Cholin. *Archiv für Dermatologische Forschung*, **240**, 107.

Keller, H.U. & Sorkin, E. (1967) Studies on chemotaxis. V. On the chemotactic effect of bacteria. *International Archives of Allergy and applied Immunology*, **31**, 505.

Keller, H.U., Hess, M.W. & Cottier, H. (1975) Physiology of chemotaxis and random motility. *Seminars in Hematology*, **12**, 47.

Krogh, H.K. & Tönder, O. (1973) Antibodies in psoriasis scales. *Scandinavian Journal of Immunology*, **2**, 45.

Langhof, H. & Müller, H. (1966) Leukotaktische Eigenschaften von Psoriasis-schuppen. *Hautarzt*, **17**, 101.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265.

Marples, R.R., Heaton, C.L. & Kligman, A.M. (1973) *Staphylococcus aureus* in psoriasis. *Archives of Dermatology*, **107**, 568.

Pinkus, H. & Mehregan, A.H. (1966) The primary histologic lesion of seborrheic dermatitis and psoriasis. *Journal of Investigative Dermatology*, **46**, 109.

Soltani, K. & Van Scott, E.J. (1972) Patterns and sequence of tissue changes in incipient and evolving lesions of psoriasis. *Archives of Dermatology*, **106**, 484.

Ward, P.A., Lepow, I.H. & Newman, L.J. (1968) Bacterial factors chemotactic for polymorphonuclear leukocytes. *American Journal of Pathology*, **52**, 725.

Ward, P.A. & Newman, L.J. (1969) A neutrophil chemotactic factor from human C′5. *Journal of Immunology*, **102**, 93.

British Journal of Dermatology (1976) 95, 9.

Carbohydrate metabolism in lichen planus

N.J.LOWE,* A.G.CUDWORTH, S.A.CLOUGH AND M.F.BULLEN

* Department of Dermatology, Liverpool Royal Infirmary, and Department of Medicine,
University of Liverpool

Accepted for publication 15 October 1975

SUMMARY

A study of forty patients with active lichen planus and a negative family history for diabetes showed that 42% had unequivocally abnormal oral glucose tolerance. The pattern of insulin response to glucose is similar to that seen in typical mild maturity-onset diabetes. There was no association between the presence of glucose intolerance and the duration or type of lesions. None of the patients with glucose intolerance had demonstrable islet-cell antibodies.

The aetiology of lichen planus is unknown. Many theories have been suggested including various metabolic disturbances (Cotton, Van Den Hurk & Vanderstaak, 1972; Black, 1972). There have been three previous reports suggesting an increased incidence of carbohydrate intolerance in this condition (Table 1). In the two recent reports in the English literature, a positive family history of diabetes was found in an appreciable proportion of cases. Furthermore, in seven of the twenty-one subjects studied by Powell *et al.* (1974) the presence of glucose intolerance was determined by the finding of a single elevated blood glucose level during a standard glucose tolerance test.

TABLE 1. Previous studies of glucose tolerance in lichen planus

Author	No. of patients	Type of lesion	Abnormal glucose tolerance
Grinspan (1966)	61	Oral	23 (37·7)*
Jolly (1972)	33	Oral	28 (85)†
Powell *et al.* (1974)	21	Cutaneous and mucosal	13 (62)‡

* Percentage in parentheses. † 21% of the total—positive F.H. of diabetes mellitus.
‡ 33% of the abnormal G.T.T. group—positive F.H. of diabetes mellitus.

The aim of the present investigation was to reassess more critically the incidence of carbohydrate intolerance in patients with active lichen planus (a) by selecting patients with no known family history of diabetes, and (b) by investigating whether there was any specific abnormality of insulin response to glucose in this condition.

PATIENTS AND METHOD

A series of unrelated patients with active lichen planus who were attending dermatological outpatients departments in Liverpool were investigated. A careful history was taken to exclude any known history of diabetes in the family, and eight patients were thus excluded. A further nine patients were excluded because they were either taking systemic steroids or declined to have a glucose-tolerance test. This left a total of forty subjects who formed the basis of the present study.

Oral glucose-tolerance testing was carried out under standard conditions following an overnight fast. Venous blood was collected at 0 (fasting) and 30, 60, 90 and 120 min after glucose.

Blood glucose was estimated using a standard automated glucose-oxidase method, and plasma was separated and stored at −20°C for the immunoassay of insulin at a later date using a modified Hales–Randle double-antibody method.

The criterion of abnormality of glucose tolerance employed was that of the Medical and Scientific Section of the British Diabetic Association (Fitzgerald & Keen, 1964), in which two or more venous blood glucose values were above the following limits: (1) A peak venous blood glucose of more than 160 mg/100 ml, plus (2) a 2-h-blood glucose of more than 110 mg/100 ml.

The age range of the patients studied was 21–74 years (mean 48·1) There were equal numbers of males and females. Particular enquiry was also made into the duration of the skin lesions. Fourteen patients had cutaneous lesions only, three mucosal lesions only, and twenty-three had both cutaneous and mucosal lesions.

As part of a larger study in which islet-cell-antibody titres have been investigated in different types of diabetes mellitus (Lendrum *et al.*, 1976), serum from the patients who had a glucose-tolerance test in the present study were also screened. The techniques, have been described elsewhere (Lendrum, Walker & Gamble 1975).

RESULTS

Of the forty subjects studied, five had clinical diabetes which had been confirmed by previous oral glucose-tolerance testing. One was on insulin therapy, and two were receiving an oral sulphonylurea drug. In four of these subjects, lichen planus had preceded the diagnosis of diabetes by between 6 months and 5 years and was still active. The patient who was insulin-dependent had been diabetic for 6 years prior to developing the skin lesions. The remaining thirty-five subjects with active lichen planus for widely varying lengths of time could be divided into three groups according to the results of the glucose-tolerance test: (1) Twelve were found to have unequivocal 'chemical diabetes', i.e. were asymptomatic but had abnormal glucose tolerance satisfying the minimum criteria listed above; (2) eighteen had unequivocally normal glucose tolerance; (3) five had a single abnormal blood glucose level and might possibly be regarded as 'borderline' chemical diabetics. In three of these, the 2-h-blood glucose level exceeded 120 mg/100 ml and the other two patients had a peak blood glucose level of 190 and 180 mg/100 ml, respectively.

The mean blood glucose and plasma insulin levels for these three groups are shown in Fig. 1. The mean ages of the 'chemical diabetic' group and the normal glucose-tolerance group were 53·4 and 41·2 years respectively. There is a significant difference between the mean ages in these two groups ($P < 0{\cdot}02$), and this suggests that variations in age may be playing a part here.

Thus a total of seventeen patients (42%) had symptomatic or chemical diabetes. If the five 'borderline' subjects are also included, the overall incidence of glucose intolerance is 55%. The pattern of insulin response to glucose in these patients is similar to that seen in typical mild maturity onset or chemical diabetes, in that there is a delayed rise in insulin levels which is sustained at 2 h (Fig. 1). The presence of glucose intolerance or delayed insulin response to glucose was not associated with obesity; most of the patients studied were of normal body weight.

In an extended study, HL-A typing was performed in a total of fifty-seven patients with lichen planus (Lowe, Cudworth & Woodrow, 1976). There was no association between the presence of demonstrable glucose intolerance and any particular HL-A phenotype.

Some of the patients in the present study were found to have glucose intolerance and lesions of lichen planus of recent onset. In contrast some subjects with chronic lesions had normal glucose tolerance. There does not appear to be any clear association between the duration of known lesions of lichen planus and the presence of chemical diabetes, although as mentioned above lichen planus had preceded the diagnosis of clinical diabetes in four of five subjects with a mild maturity-onset type of diabetes. There was found to be no significant association between the presence of mucosal lichen planus and glucose intolerance as had been inferred previously (Grinspan *et al.*, 1966).

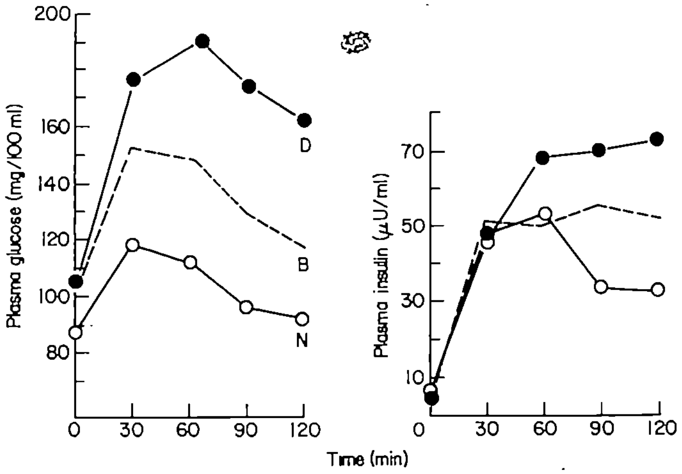


FIGURE 1. Mean plasma glucose and mean plasma insulin levels in (D) patients with overt glucose intolerance (n = 12); (B) borderline diabetics (n = 5), and (N) in the group with normal glucose tolerance (n = 18).

The presence of islet-cell antibodies was sought in the sera of the thirty-five subjects who had had a recent glucose-tolerance test. None of the subjects who were found to have chemical diabetes had a positive islet-cell-antibody titre, whereas two subjects with unequivocally normal glucose tolerance had demonstrable antibody although in low titre. The significance of this finding is unknown. It is not clear at present what is the incidence of islet-cell antibodies in a normal adult population.

DISCUSSION

The incidence of symptomatic plus chemical diabetes in the present study of forty unrelated randomly chosen hospital patients with active lichen planus is 42%. Although simple enquiry into the family history for the presence or absence of diabetes is not completely reliable, nevertheless, it is of interest that the incidence of carbohydrate intolerance in the present study is considerably less than in two recently reported series in which there was an appreciable known incidence of a positive family history for diabetes (Table 1). The object of selecting patients with a negative family history was to reduce the possible effect of diabetogenic genes being present which might have increased the overall incidence of glucose intolerance.

Problems also arise in any study of this nature because of the differences in criteria which are used to assess the presence of glucose intolerance (Valleron *et al.*, 1975). Thus, the calculated incidence for the present series using the criteria employed by Jolly (1972) and Powell *et al.* (1974), would be 55 and 52·5%, respectively. Conversely, taking the data of Powell *et al.*, if the subjects with a positive family history for diabetes are excluded and if the same criterion for abnormal glucose tolerance as used in the present analysis is employed, the incidence of unequivocal diabetes is only 17%, and if the 'borderline' chemical diabetic subjects are included, the incidence rises to 50%. The estimated prevalence rate of carbohydrate intolerance in the Bedford survey (Butterfield, 1964) was 12–14%. Thus the incidence in the present study is at least 3 times greater than this, and confirms that there is a definite association between lichen planus and glucose intolerance of a predominantly asymptomatic type.

The nature of the association between the two conditions remains entirely speculative. In four of five subjects with symptomatic diabetes, lichen planus had preceded the clinicial diagnosis. This might suggest that lichen planus is a similar phenomenon to necrobiosis lipoidica diabeticorum which generally antedates the onset of diabetic symptoms, and is considered by many to be prognostic of diabetes. However, there seems to be little, if any, similarity between the two skin lesions.

Recently, reduced levels of IgA and IgM have been reported in lichen planus with the suggestion that these patients have an increased susceptibility to viral infection (Stankler, 1975). It is unlikely that virus infection is the cause of diabetes in this condition. The nature of the diabetes is generally a very mild form of disturbance similar to that seen in typical mild maturity-onset diabetes. Furthermore, the pattern of insulin response is indistinguishable from that seen in mild maturity-onset diabetics, exhibiting a delayed elevation in peripheral plasma insulin levels in association with prolonged hyperglycaemia (Seltzer *et al.*, 1967). An absence of islet-cell-antibody titres in the chemical diabetics in this study is in marked contradistinction to early juvenile-onset insulin-dependent diabetes (Lendrum *et al.*, 1975), in which the circumstantial evidence for viral infection is much stronger (Steinke & Taylor, 1974).

The basic question whether lichen planus like necrobiosis lipoidica diabeticorum is an early marker of developing diabetes in a small percentage of patients, or whether some immunological disturbance associated with the development of lichen planus predisposes to insulin resistance and subsequent mild diabetes, cannot be answered. Compared with necrobiosis lipoidica diabeticorum, it is often a much more transient condition. Moreover, it is not known whether or not the detectable abnormality in glucose handling is also a transient phenomenon. In order to throw further light on this important question, long-term studies are currently being carried out on some of the patients in the present study.

ACKNOWLEDGMENTS

We are grateful to our colleagues who allowed us to study their patients, and to Dr R. Lendrum for performing the the islet-cell antibody studies.

REFERENCES

BLACK, M.M. (1972) The pathogenesis of lichen planus. *British Journal of Dermatology*, **86**, 302.

BUTTERFIELD, W.J.H. (1964) Summary of the Bedford diabetes survey. *Proceedings of the Royal Society of Medicine*, **57**, 196.

COTTON, D.W.K., VAN DEN HURK, J.J.M.A. & VANDERSTAAK, W.B.J.M. (1972) Lichen planus; an inborn error of metabolism. *British Journal of Dermatology*, **87**, 341.

FITZGERALD, M.G. & KEEN, H. (1964) Diagnostic classification of diabetes. *British Medical Journal*, **i**, 1568.

GRINSPAN, D., DIAZ, J., VILLAPOL, L.O., SCHNEIDERMAN, J., BORDICHESKY, R., PALESE, D. & FAERMAN, J. (1966) Lichen ruber planus de la muguese buccale, son association a un diabete. *Bulletin de la Société française de dermatologie et de syphiligraphie*, **73**, 898.

HALES, C.N. & RANDLE, P.J. (1963) Immunoassay of insulin with insulin-antibody precipitate. *Biochemical Journal*, **88**, 137.

JOLLY, M. (1972) Lichen planus and its association with diabetes mellitus. *Medical Journal of Australia*, **1**, 990.

LENDRUM, R., WALKER, G. & GAMBLE, D.R. (1975) Islet cell antibodies in juvenile diabetes mellitus of recent onset. *Lancet*, **i**, 880.

LENDRUM, R., WALKER, G., CUDWORTH, A.G., WOODROW, J.C. & GAMBLE, D.R. (1976) HL-A linked genes and the presence of islet cell antibody. (To be published)

LOWE, N.J., CUDWORTH, A.G. & WOODROW, J.C. (1976) HL-A antigens in lichen planus. *British Journal of Dermatology*, (to be published).

POWELL, S.M., ELLIS, J.P., RYAN, T.J. & VICKERS, H.R. (1974) Glucose tolerance in lichen planus. *British Journal of Dermatology*, **91**, 73.

SELTZER, H.S., ALLEN, W., HERRON, A.L. JR & BRENNAN, M.T. (1967) Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *Journal of Clinical Investigation*, **46**, 323.

STANKLER, L. (1975) Deficiency of circulating IgA and IgM in adult patients with lichen planus. *British Journal of Dermatology*, **93**, 25.

STEINKE, J. & TAYLOR, K.W. (1974) Viruses and the aetiology of diabetes. *Diabetes*, **23**, 631.

VALLERON, A.J., ESCHWÈGE, E., PAPOZ, L. & ROSSELIN, G.E. (1975) Agreement and discrepancy in the evaluation of normal and diabetic oral glucose tolerance test. *Diabetes*, **24**, 585.

British Journal of Dermatology (1976) 95, 13.

Excision repair of DNA in some photodermatoses

M.ICHIHASHI* AND C.A.RAMSAY†

Department of Photobiology, Institute of Dermatology, London E9

Accepted for publication 10 October 1975

SUMMARY

Excision repair of DNA was measured in a number of photosensitive subjects by estimating unscheduled DNA synthesis in cultured fibroblasts after ultraviolet radiation. The amount of unscheduled DNA synthesis and the action spectrum for this process were estimated in each subject and compared with seven normal subjects.

Two subjects with polymorphic light eruption and three with actinic reticuloid exhibited normal levels of DNA repair and normal action spectra. In one subject with classical xeroderma pigmentosum no excision repair could be demonstrated whilst in another the amount of repair was lower than normal.

The finding of normal DNA excision repair in polymorphic light eruption does not support an earlier claim that this repair process is abnormal in this common photodermatosis.

When deoxyribonucleic acid (DNA) is damaged by ultraviolet radiation (UVR) one important photoproduct is the pyrimidine, principally thymine, dimer. One method of reversing UVR-induced damage is by the process of excision repair whereby the dimer is removed from the DNA chain and new bases inserted to produce continuity of the chain (Cleaver, 1970). Although fibroblasts grown from skin biopsies are the cells usually used for the investigation of excision repair in mammals, it is a process which is present in other tissues and can be demonstrated in lymphocytes (Burk *et al.*, 1971), in epidermal cells (Robbins, Levis & Miller, 1972) and directly in the skin with an *in vivo* technique (Epstein *et al.*, 1970).

In 1968 Cleaver reported that excision repair was absent or markedly reduced in fibroblasts from patients with the photosensitive disease xeroderma pigmentosum (XP) and this was the first report of a mammalian cell line with an abnormality in this type of DNA repair. Although the majority of patients with XP show the abnormality of excision repair, there are some patients who have what appears to be the same disease clinically but in whom excision repair is normal (Robbins *et al.*, 1972; Cleaver, 1972; Ramsay & Giannelli, 1975).

Normal excision repair has been demonstrated in fibroblasts from patients with a number of other diseases, some showing sensitivity to sunlight (Cleaver, 1970; Epstein, Fukuyama & Epstein, 1971). It

---

Present addresses: * Department of Dermatology, University of Kobe School of Medicine, Ikuta-Ku, Kobe, Japan. † Department of Dermatology, Toronto Western Hospital, Toronto, Ontario, Canada.

appeared, therefore, that this abnormality in DNA repair was limited to the majority of patients with XP, but then Horkay and her colleagues (1973) reported very low levels of excision repair after UVR in lymphocytes of six patients with polymorphic light eruption (PLE) as well as in patients with XP. These same authors showed that lymphocytes from patients with porphyria cutanea tarda exhibited normal repair.

We therefore decided to investigate DNA excision repair in patients with some photodermatoses including PLE.

MATERIALS AND METHODS

Subjects

There were two patients with PLE, three with actinic reticuloid (Ive *et al.*, 1969) and two with XP both of whom have been reported previously (Ramsay & Giannelli, 1975; Cases 3 and 4). The normal subjects were seven adults: 5 male, 2 female (Ichihashi & Ramsay, 1975).

Methods

Skin biopsies were obtained from the medial aspect of the upper arm and fibroblasts cultures obtained. The culture medium was Eagle's minimal essential medium containing penicillin (100 u/ml) and streptomycin (100 μg/ml) supplemented with 10–20% fetal calf serum. To provide monolayers of cells, cultures were trypsinized and grown in small plastic Petri dishes containing 2 glass coverslips at 37°C, in air with 2–50% CO_2, for up to 7 days.

Irradiation was by means of a water prism monochromator and 2 kW xenon arc source (Magnus *et al.*, 1959) and intensity of radiation was measured with a vacuum thermopile and digital voltmeter. Wavebands between 260 and 320 nm were selected and at each waveband a dose of 10 mJ/cm^2 was given. In addition a broad band UVR source (320–400 nm) was used (Ramsay & Obreshkova, 1974). Immediately prior to irradiation the medium in the Petri dish was removed and the culture washed once with Hanks' solution containing phenol red as an indicator. After irradiation new medium was added and cultures incubated at 37°C for 30 min.

Radioactive labelling of cells

Thirty min after irradiation the medium was removed and fresh medium containing 5 μCi/ml of methyl ^{3}H thymidine (specific activity 15–30 Ci/mmol, Radiochemical Centre, Amersham) was added. After 3 h incubation the cultures were washed with cooled Hanks's solution and saline, fixed with glacial acetic acid: ethanol (1:3v/v) for 15 min and dried.

Autoradiography

The cover slips were coated with Ilford K2 emulsion in gel form diluted 50:50 with 2% glycerol and after 7–10 days exposure developed and stained with haematoxylin.

Control cultures were treated in exactly the same way except that they were not irradiated.

Assessment of unscheduled DNA synthesis

Two hundred cells were counted and the cells divided into five groups according to the number of grains over each nucleus: Group A (0–5 grains); B (6–10); C (11–20); D (21–50); E (>50). Cells in Groups A were regarded as showing background labelling and those in Group E as showing scheduled or S phase DNA synthesis. In control unirradiated cultures some background labelling was seen but apart from this the only heavily labelled cells were those in S phase. These cells always contained more than 50 grains per nucleus. The total number of grains in cells of Groups B–D was calculated and the difference between this total in irradiated and control cells taken as the level of unscheduled DNA synthesis at each wavelength.

RESULTS

The results are shown in Fig. 1 where the mean value for unscheduled DNA synthesis at each waveband is plotted for each disease and for the normal subjects. With the XP patients the results are given for each case separately. The values for normal subjects are those reported by Ichihashi & Ramsay (1975).

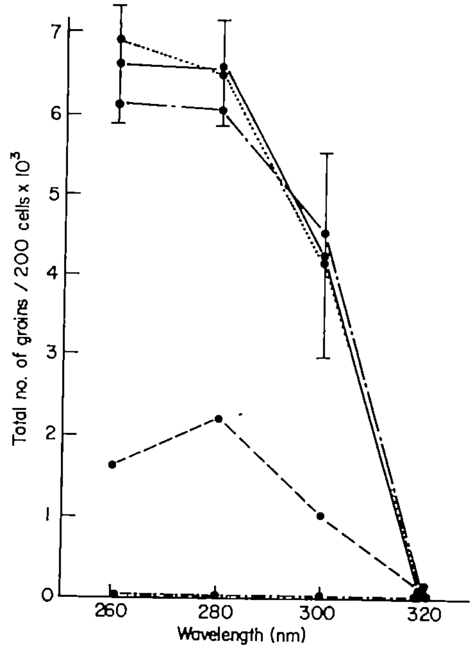


FIGURE 1. Action spectrum for unscheduled DNA synthesis in cultured fibroblasts. Each point represents the mean for the normal subjects and for each disease except for xeroderma pigmentosum where the results for each case are plotted separately. The bars represent the standard deviations for the normal subjects.—— Normal subjects; .... polymorphic light eruption; —·— actinic reticuloid; – – – – xeroderma pigmentosum; —·—·— xeroderma pigmentosum.

Polymorphic light eruption

Irradiation of the skin of these patients with narrow waveband UVR and visible light *in vivo* showed abnormal reactions limited to the 300–320 nm part of the spectrum. Fibroblasts from these patients showed normal levels of unscheduled DNA synthesis after UVR and the action spectrum was also normal.

Actinic reticuloid

The skin of all three patients was very sensitive to both 300–320 nm UVR and to long-wave UVR *in vivo*. The level of excision repair in fibroblasts from these patients was quite normal and the action spectrum for this was also normal. Despite the fact that these patients showed sensitivity of their skin to long-wave UVR no unscheduled DNA synthesis was detected in the fibroblasts after irradiation with doses of long-wave UVR (320–400 nm) up to 5 J/cm^2.

Xeroderma pigmentosum

One patient showed much lower levels of unscheduled DNA synthesis than the normal controls. The action spectrum was also different from normal in that there appeared to be greater reactivity at 280 nm than at 260 nm. However, it is emphasized that this result was obtained on only one patient and it is therefore not possible to state whether the apparent difference between 260 and 280 nm is significant.

Unscheduled DNA synthesis could not be detected at any waveband with the dose in the other patient with XP. Fibroblasts from this patient have been examined elsewhere (Ramsay & Giannelli, 1975, Case 4) and very low levels of unscheduled synthesis were detected when a low pressure mercury lamp emitting predominantly at 254 nm was used as the irradiation source.

DISCUSSION

We were unable to detect any abnormality in DNA excision repair in our two patients with PLE and this contrasts sharply with the results of Horkay *et al.* (1973) who reported markedly reduced levels of repair in six patients with PLE. It must be noted that we used fibroblasts whereas Horkay *et al.* used lymphocytes and the methods of assessing repair were different. Horkay *et al.* measured the rate of incorporation of radioactivity with a scintillation counter after incubating the lymphocytes in tritiated thymidine, whilst we used an autoradiographic method.

The difference in the tissue used for experiments on DNA excision repair should not be important if we can extrapolate from the results with various tissues from patients with XP. In this disease the abnormality in excision repair exists not only in fibroblasts where it was first detected (Cleaver, 1968) but also in epidermal cells (Robbins *et al.*, 1972) and in lymphocytes (Burk *et al.*, 1971). If a similar abnormality is present in patients with PLE one would expect the defect to exist in other tissues as well as lymphocytes.

Our two patients with PLE gave a typical history with a delay of several hours between exposure to sunlight and the onset of their rash. They both showed abnormal erythemato-papular reactions of their skin when it was irradiated with narrow waveband in the 290–320 nm part of the spectrum. DNA excision repair in these two patients was quite normal both with respect to the level of repair and also to the action spectrum for repair (Fig. 1). At present we cannot explain the difference between our results and those of Horkay *et al.* (1973) except to suggest that the abnormality in excision repair in PLE reported by these other authors may not be present in all patients with PLE. Further studies on this disease are obviously necessary.

Excision repair was completely normal in our three patients with actinic reticuloid. In this disease sensitivity of the skin to both short-wave and long-wave UVR is characteristic and many of these patients also show sensitivity to visible light (Ive *et al.*, 1969). However, the action spectrum for repair was normal and no repair could be induced in our patients by long-wave UVR with doses up to 5 J/cm^2.

Despite the differences in the UVR sensitivity of the skin of these patients with PLE and actinic reticuloid the action spectrum for repair in their fibroblasts was virtually identical (Fig. 1) and in particular no repair could be induced by long-wave UVR in those patients with actinic reticuloid whose skin did show long-wave UVR sensitivity. It seems very likely that the molecular changes which accompany excision repair of DNA bear no relationship to the erythematous and oedematous reactions of the skin which follow exposure to UVR in this photodermatosis.

In one patient with XP no repair could be detected with our method. In the other patient with XP the amount of DNA repair was reduced when compared with the normal subjects. The findings in these patients were as expected in this disease. The action spectrum for repair in one of the XP patients was not exactly the same shape as normal at the dose used (10 mJ/cm^2). Repair seemed to be stimulated more by 280 nm than by 260 nm in the XP patient whilst in the normals 280 and 260 nm seemed to be equally effective. The significance of this finding is uncertain at present. Until we have more information on the action spectrum for DNA excision repair in other patients with XP it is not possible to say whether this difference between 260 and 280 nm is significant.

It has recently been reported that patients with XP who have normal excision repair of DNA ('XP variants') do show an abnormality in post-replication repair (Lehmann *et al.*, 1975). The present investigation was concerned solely with excision repair as demonstrated by unscheduled DNA synthesis. It is quite possible that patients with photodermatoses other than XP may show abnormalities in other DNA repair processes.

REFERENCES

BURK, P.G., LUTZNER, M.A., CLARKE, D.C. & ROBBINS, J.H. (1971) Ultraviolet stimulated thymidine incorporation in xeroderma pigmentosum lymphocytes. *Journal of Laboratory and Clinical Medicine*, **77**, 759.

CLEAVER, J.E. (1968) Defective repair replication of DNA in xeroderma pigmentosum. *Nature*, **218**, 652.

CLEAVER, J.E. (1970) DNA damage and repair in light-sensitive human skin disease. *Journal of Investigative Dermatology*, **54**, 181.

CLEAVER, J.E. (1972) Xeroderma pigmentosum: Variants with normal DNA repair and normal sensitivity to ultraviolet light. *Journal of Investigative Dermatology*, **58**, 124.

EPSTEIN, J.H., FUKUYAMA, K., REED, W.B. & EPSTEIN, W.L. (1970) Defect in DNA synthesis in skin of patients with xeroderma pigmentosum demonstrated *in vivo*. *Science*, **168**, 1477.

EPSTEIN, W.L., FUKUYAMA, K. & EPSTEIN, J.H. (1971) Ultraviolet light, DNA repair and skin carcinogenesis in man. *Federation Proceedings*, **30**, 1766.

HORKAY, I., TAMASI, P. & CSONGOR, J. (1973) UV light induced DNA damage and repair in lymphocytes in photodermatoses. *Acta dermato-venereologica*, **53**, 105.

ICHIHASHI, M. & RAMSAY, C.A. (1975) The action spectrum and dose response studies of unscheduled DNA synthesis in normal human fibroblasts. *Photochemistry and Photobiology*, in press.

IVE, F.A., MAGNUS, I.A., WARIN, R.P. & WILSON JONES, E. (1969) 'Actinic reticuloid', a chronic dermatosis associated with severe photosensitivity and the histological resemblance to lymphoma. *British Journal of Dermatology*, **81**, 469.

LEHMANN, A.R., KIRK-BELL, S., ARTLETT, C.F., PATERSON, M.C., LOHMAN, P.H.M., DE WEERD-KASTELEIN, E.A. & BOOTSMA, D. (1975) Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV irradiation. *Proceedings of the National Academy of Science*, **72**, 219.

MAGNUS, I.A., PORTER, A.D., MCCREE, K.J., MORELAND, J.D. & WRIGHT, W.D. (1959) A monochromator. An apparatus for the investigation of the responses of the skin to ultraviolet, visible and near infra-red radiation. *British Journal of Dermatology*, **71**, 261.

RAMSAY, C.A. & OBRESHKOVA, E. (1974) Photosensitivity from nalidixic acid. *British Journal of Dermatology*, **91**, 523.

RAMSAY, C.A. & GIANNELLI, F. (1975) The erythemal action spectrum and deoxyribonucleic acid repair synthesis in xeroderma pigmentosum. *British Journal of Dermatology*, **92**, 49.

ROBBINS, J.H., LEVIS, W.R. & MILLER, A.E. (1972) Xeroderma pigmentosum epidermal cells with normal UV-induced thymidine incorporation. *Journal of Investigative Dermatology*, **59**, 402.

British Journal of Dermatology (1976) **95**, 19.

A follow-up study of patients with recurrent urticaria and hypersensitivity to aspirin, benzoates and azo dyes

ANN-MARI ROS, LENNART JUHLIN AND GERD MICHAËLSSON

Department of Dermatology, University Hospital, Uppsala, Sweden

Accepted for publication 17 October 1975

SUMMARY

We have studied seventy-five patients with recurrent urticaria and angio-oedema of more than 4 months duration and with positive provocation tests to aspirin, azo dyes, and/or benzoates. Cross-reactions between the test compounds were common. The patients were recommended to be on a diet free from salicylates, benzoates, and azo dyes. They were then followed for 6–24 months. At the follow-up, 24% were free from symptoms, 57% considered themselves much better and 19% stated that they were slightly better or unchanged. All patients had followed the diet for at least 1–3 months. Most of those who became totally free of symptoms did not continue with the diet, while most of the patients who considered themselves much better found that it was necessary to continue on the recommended diet. They usually developed symptoms as soon as they ingested something containing azo dyes or benzoates. To be able to maintain such a diet, it is important that the content of additives in food and drugs be properly declared.

Benzoates and azo dyes are commonly used as additives in food and drugs. These compounds, as well as aspirin, are often responsible for flare-ups in patients with recurrent urticaria (Michaëlsson & Juhlin, 1973). The mechanism for this hypersensitivity is obscure and no evidence for an antigen–antibody mediated reaction has been found (see Warin & Champion, 1974). Provocation tests that precipitate an urticarial reaction are still the only way to establish the hypersensitivity. Patients with positive reactions have been asked to try to follow a diet free from these substances. In order to determine to what extent they improve on such a regimen, a follow-up study has been done on seventy-five patients, who, 6 months–2 years previously, had one or several positive provocations to tests with aspirin, benzoates and/or azo dyes.

METHODS

Patients

The study included 24 men and 51 women between the ages of 14 and 66 years with one or several positive provocation tests to aspirin, benzoates, or azo dyes. They represent 68% of all patients with chronic urticaria studied during this period. Twenty-one had symptoms of recurrent urticaria and fifty-four had urticaria and recurrent angio-oedema periodically or continuously for at least 4 months. The frequency of symptoms was noted both before the provocation tests and at follow-up after 6–24 months. The patients were asked whether they had observed any flare-up of urticaria after taking aspirin or after ingestion of food known to contain a high content of preservatives and/or dyes. None had physical urticaria or any evident allergic urticaria. Eight of the patients also had bronchial asthma and five had thyroid disorders that were being treated with l-thyroxine. One patient had diabetes mellitus, one had pernicious anaemia, one hyperparathyroidism, and one systemic lupus erythematous. The patients were otherwise healthy. They had previously been treated with different antihistamines, mostly without any obvious improvement.

The provocation tests have previously been described in detail (Michaëlsson & Juhlin, 1973). The patients were admitted to the hospital and were given a diet free from dyes and preservatives. Those who had frequent recurrences of urticaria often had been on such a diet even before being admitted. The provocation tests were performed when the patients had slight or no symptoms of urticaria. Antihistamines or steroids were not allowed. As a positive provocation, only objective signs occurring within 20 h were accepted, i.e. urticaria, angio-oedema, rhinitis, sneezing, or hoarseness. Subjective symptoms such as itchy skin, headache, breathing difficulties, etc. were recorded but not accepted as a positive provocation.

The following compounds and doses were routinely tested: Placebo = lactose or starch (100 mg), aspirin (1, 5, 10, 50, 500, 1000 mg), 4-hydroxybenzoic acid (50, 100 mg), sodium benzoate (50, 250, 500 mg), and the azo dyes, tartrazine, New Coccine, and Sunset Yellow (1, 2, 5, 10 mg). Patients with a history of asthma started with 0·1 mg of aspirin or dye. After a light breakfast, the substance to be tested was given in the lowest dose at 8 a.m. If no objective reaction could be noted, additional and increased doses were given at 1 h intervals. Only one substance was given per day. The tests which were questionable and difficult to interpret were repeated on another day. The order of the tests varied but placebo was usually given at the beginning and aspirin at the end.

The patients were interviewed 6 months–2 years after the provocation tests. During this time they had been asked to follow a diet that was free from dyes and benzoates as far as possible and to avoid drugs containing aspirin and those that were coloured. They had been given written general instructions with concrete examples about what to eat and what to avoid (see Michaëlsson & Juhlin, 1973).

RESULTS

Provocation tests

The types of drugs giving positive reactions are shown in Table 1. Twelve of the patients tested were positive only to aspirin, two patients only to benzoates, and seven patients only to azo dyes. Positive provocations to several compounds were common. Those who were positive to aspirin often also reacted to the other compounds. Sixteen patients were negative to aspirin but positive to benzoates and/or azo dyes. Provocations with the placebo were not done with two patients but were negative in the other seventy-three patients.

Twenty-seven of the fifty-nine patients with positive aspirin provocation had previously observed a flare-up of urticaria after taking aspirin (Table 1). One patient with a history of hypersensitivity to aspirin had a negative aspirin provocation test.

Thirteen of the sixty-three patients with positive provocations to benzoates and/or azo dyes had a positive history to these compounds. Three patients with a history of reaction to preservatives and/or dyes had negative provocations to these compounds but were positive to aspirin.

Nineteen per cent of the patients with only one positive provocation had a history of hypersensitivity, whereas 54% of those with more than one had such a history.

TABLE 1. Number of patients with positive provocations and a history of hypersensitivity to aspirin, benzoates, and azo dyes

Substance	Positive provocations	History of hypersensitivity	
		Aspirin	Benzoates and azo dyes
Aspirin only	12	3	0
Benzoates only	2	0	0
Azo dyes only	7	0	1
Benzoates + azo dyes	7	0	3
Aspirin + benzoates	11	7	1
Aspirin + azo dyes	12	7	3
Aspirin + benzoates + azo dyes	24	10	5
Totals	75	27	13

Follow-up study

All of the seventy-five patients were interviewed after 6–24 months. The patient's record of the number of days when urticaria occurred (daily, a few times a week, or 1–4 times per month) during the month prior to admission to hospital for provocations is compared with the corresponding figure for the month before the follow-up (Fig. 1). Forty-nine patients were completely free from urticaria or had urticaria less frequently compared with the previous hospital admission, while eight patients had urticaria more frequently. It should be pointed out that here only the frequency of urticaria is recorded. The intensity of the symptoms and the number of lesions are evaluated when the patients give their opinion of the changes in their condition after the diet (Table 2). Of the seventy-five patients, eighteen had

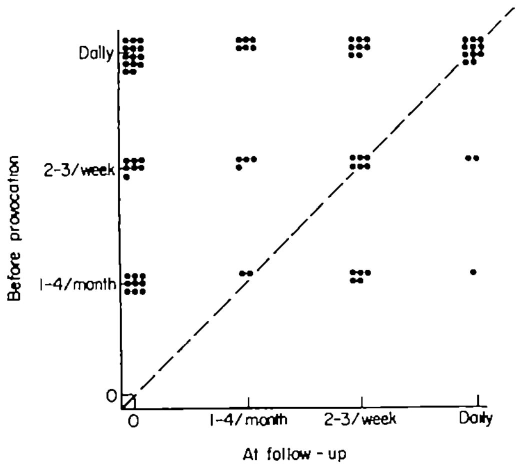


FIGURE 1. Frequency of attacks with urticarial lesions during the month prior to provocation tests and the follow-up after 6–24 months. Frequencies are based on the patient's statement of approximate number of days with urticaria. Each dot represents a patient.

TABLE 2. Follow-up study of seventy-five patients with chronic urticaria in relation to diet maintenance

Patient's opinion of urticaria status	Diet 1–3 months	Maintains diet	Totals
Symptom-free	13	5	18
Much better	11	32	43
Slightly better or unchanged	3	11	14
Totals	27	48	75

C

P, 25,466.

TABLE 3. Status at follow-up and number of types of compounds (aspirin, azo dyes, benzoates) giving positive provocations

Patients' opinions of urticaria status	No. of types 1	2	3	Totals
Sympton-free	7	8	3	18
Much better	12	18	13	43
Slightly better or unchanged	2	4	8	14
Totals	21	30	24	75

TABLE 4. Duration and improvement of urticaria

Duration of urticaria (years)	Free from symptoms	Much better	Slightly better/ unchanged	Totals
<½	10	9	3	22
½–1	1	7	2	10
1–5	2	15	6	23
>5	5	12	3	20
Totals	18	43	14	75

been free from urticaria for at least 6 months and forty-three patients considered themselves much better. Fourteen patients thought that they were either slightly better or unchanged.

The diet was followed for at least 1–3 months by all patients and forty-eight were still on it at follow-up. One of the eighteen patients who were free from urticaria for 6 months still followed the diet since he dared not give it up. Four others who were symptomless had a modified diet and avoided aspirin and certain products with a high content of dyes or benzoates.

Thirty-two of the forty-three patients who considered themselves 'much better' still followed the diet and their urticaria worsened if they failed to do so. The eleven patients of the forty-three who had followed the diet only for a short time did not consider the diet of any significance with regard to their urticarial symptoms but some avoided aspirin and strongly coloured products.

Fourteen patients were unchanged and eleven of them still followed the diet; several of them became worse if they did not.

In Table 3 the follow-up status of the patients has been correlated with the number of types of compound giving positive provocations (aspirin, benzoates, or azo dyes). Of the eighteen patients free from urticaria, seven had positive provocation to only one compound and three were positive to three types. Among the fourteen patients who were unchanged or slightly better, two were positive to one compound and eight to three.

The duration of urticaria in the seventy-five patients was over 5 years in twenty patients and five of them were symptom-free at follow-up (Table 4). Twenty-two patients had suffered from urticaria for less than a year and eleven of these were symptom-free at follow-up.

COMMENT

Fifty-five per cent of all our patients with chronic urticaria and 84% of those with positive provocation tests had reactions to aspirin; however, only about half of those reacting knew that they had such a hypersensitivity. Similar figures for reactions to aspirin and food additives in patients with bronchial asthma have been found by Rosenhall & Zetterström (1974). Our figures concerning reactions to aspirin are somewhat higher than those reported in the literature, where an exacerbation was noted after aspirin challenge in 41% of the patients with chronic urticaria (James & Warin, 1970) and where a positive history was found in about 22% of them (Moore-Robinson & Warin, 1967; Champion *et al.*, 1969). Lower figures for reactions to tartrazine (13–25%) and benzoates (11–40%) have been reported by others (Doeglas, 1975; Warin & Smith, 1976; Thune & Granholt, 1976). The reason for the higher figures in our patients might be due to the fact that they were not given any antihistamines and the possibility that they were in a more active phase of their disease. In addition to tartrazine, we also tested two other azo dyes and the enlarged azo group may have yielded a higher percentage of positive reactions. Another important factor is that our patients at the beginning of our studies were more selected than those in the other studies since twelve of them were referred to us because they had a history of aspirin hypersensitivity and recurrent urticaria without improvement after withdrawal of aspirin. From the high frequency of reactions to aspirin, it seems obvious that patients with urticaria should avoid this drug even if they do not have a history of worsening after aspirin.

Our earlier findings that cross-reactions between azo dyes and benzoates were common has been verified (Michaëlsson & Juhlin, 1973). Samter & Beers (1967) and Settipane & Pudupakkam (1975) reported a cross-reactivity rate to tartrazine of 8 and 15% respectively in aspirin–hypersensitive patients with symptoms of mainly asthma and rhinitis. Details of the test method were given by Settipane & Pudupakkam, who used a test dose of 0·44 mg of tartrazine and judged the reaction within 2 h. Most of our patients reacted later and to higher doses, which might explain why cross-reactivity was more common in our patients. From the present investigation, however, it is also evident that hypersensitivity to dyes or benzoates without any reaction to aspirin is not uncommon and occurred in sixteen of the seventy-five patients. A history of hypersensitivity to dyes and benzoates was obtained in 21% of the patients with positive provocations to these compounds. That the figure is lower than that for aspirin is not surprising as the additives are more or less hidden in a number of drugs and food products, whereby their intake may easily be overlooked.

The judgement of whether a reaction is positive or negative is not always easy and, in many cases, repeated provocations with placebo and additives are necessary. If the patients are in an acute stage with urticaria every day, it is impossible to continue the tests. The evaluation of the provocations must be done carefully with detailed annotations of signs and symptoms. The symptoms occur in most patients after 6–12 h but might appear as late as 20 h after provocation. Symptoms of severe facial angio-oedema mainly occurred after aspirin provocation and were more easily evaluated than a few urticarial weals.

Some patients react only with a number of subjective symptoms such as headache, tiredness, joint pains, irritability, etc. We have hitherto considered these results as probably negative although this may not be correct if repeated placebo provocations are not associated with any symptoms. For further evaluation of the relevance of such symptoms, studies of the reactions in healthy controls, as well as in patients with other skin disorders, are being undertaken.

The patients with positive provocations have been recommended a diet free from aspirin and food additives. To evaluate its effect, our results on follow-up must be compared with the expected duration of symptoms without any treatment. The total duration of urticaria of unknown aetiology has been calculated by Champion *et al.* (1969). When all cases with allergic, cholinergic and physical urticaria had been removed, they had a material of 438 cases with urticaria for at least 1 month. The prognosis was best for the patients with only urticaria and without angio-oedema. If their urticaria had lasted for 4 months, it was expected that about 23% should be free from symptoms after 12 months. The number of symptom-free patients in our study seems to follow their expectancy curves for urticaria with the exception that we have some patients with urticaria for more than 10 years who became symptom-free. Thus, many of the patients in our study who became totally symptom-free probably would have been so even without the diet. In some, there was a striking association between decrease of symptoms and start of diet. However, the group of patients from whom the effect of the diet could best be judged were those who claimed that they were much better, which means that they had only minor and occasional symptoms. After 3 months, some of them found that they had to avoid aspirin or drinks and candies that were obviously coloured. but that it was not otherwise necessary to keep to the diet. This confirms the views of Warin (1960) that many of these patients benefit by just avoiding aspirin and is also in agreement with the findings of Warin & Smith (1975) that some patients may gradually relax their diet. Other patients found that it was necessary to be on the recommended diet. They developed weals as soon as they took something containing azo dyes or benzoates. The personal history of these patients makes it clear that it really is important for them to avoid these substances and that the diet is of great value. The results in these patients with their pronounced improvement were encouraging. Preliminary results of a similar study were recently reported by Warin & Smith (1975). They found that thirty-five of forty-five patients cleared or improved considerably and in three patients there was no effect, while five were impossible to assess. The effect of diet seems to agree well with the results presented here, but a direct comparison is difficult since they also included yeasts and penicillin in their study.

The patients were asked about the number of urticarial attacks during the month before the provocations and before the follow-up. Sixty-four per cent reported less frequent urticarial attacks at follow-up. Such information, however, says nothing about the severity, extension, and itching of the lesions. These factors are involved in the patient's subjective opinion of his urticaria and this probably explains why as many as 81% of the patients consider themselves symptom-free or much better.

As expected, there are patients who have no significant improvement despite trying to avoid aspirin, as well as dyes and benzoates. Some of them may have cross-reactions to similar naturally occurring substances or have later been found to be sensitive to other additives. For some patients other factors might be of greater importance.

To maintain the diet is fairly simple if the contents of foods and drugs are properly declared. If they are not, it is certainly more difficult. It seems reasonable that full information on products should be given in order to make a free choice possible. The need for this is becoming increasingly evident.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council (Project No. B 73-19X-769-08C), the Welander and Finsen Foundations and the Swedish Life Insurance Companies Research Board.

REFERENCES

CHAMPION, R.H., ROBERTS, S.O.B., CARPENTER, R.G. & ROGER, J.H. (1969) Urticaria and angio-oedema. A review of 554 patients. *British Journal of Dermatology*, **81**, 588.

DOEGLAS, H.M.G. (1975) Reactions to aspirin and food additives in patients with chronic urticaria, including the physical urticarias. *British Journal of Dermatology*, **93**, 135.

JAMES, J. & WARIN, R.P. (1970) Chronic urticaria: The effect of aspirin. *British Journal of Dermatology*, **82**, 204.

MICHAËLSSON, G. & JUHLIN, L. (1973) Urticaria induced by preservatives and dye additives in food and drugs. *British Journal of Dermatology*, **88**, 525.

MOORE-ROBINSON, M. & WARIN, R.P. (1967) Effect of salicylates in urticaria. *British Medical Journal*, **iv**, 262.

ROSENHALL, L. & ZETTERSTRÖM, O. (1974) Personal communication.

SAMTER, M. & BEERS, R.J.Jr. (1967) Concerning the nature of intolerance to aspirin. *Journal of Allergy*, **40**, 281.

SETTIPANE, G.A. & PUDUPAKKAM, R.K. (1975) Aspirin intolerance. III. Subtypes, familial occurrence and cross-reactivity with tartrazine. *Journal of Allergy and Clinical Immunology*, **56**, 215.

THUNE, P. & GRANHOLT, A. (1976) Provocation tests with antiphlogistica and food additives in recurrent urticaria. *Dermatologica*, in press.

WARIN, R.P. (1960) The effect of aspirin in chronic urticaria. *British Journal of Dermatology*, **72**, 350.

WARIN, R.P. & CHAMPION, R.H. (1974) *Urticaria*. Vol. 1 in Major Problems in Dermatology. Saunders, London.

WARIN, R.P. & SMITH, R.J. (1975) Challenge test battery in chronic urticaria. *British Journal of Dermatology*, **93**, Suppl. 11, 19.

WARIN, R.P. & SMITH, R.J. (1976) Challenge test battery in chronic urticaria. *British Journal of Dermatology*, **94**, 401.

British Journal of Dermatology (1976) **95**, 25.

The role of divalent cations in epidermolysis

ROBERT L. DIMOND,* KENT L. ERICKSON† AND KIRK D. WUEPPER

Department of Cutaneous Biology, Oregon Regional Primate Research Center, Beaverton, Oregon 97005, and Department of Dermatology, Medical School Division, University of Oregon Health Sciences Center, Portland, Oregon 97201, U.S.A.

Accepted for publication 9 October 1975

SUMMARY

In experiments designed to elucidate the role of divalent cations in maintaining the integrity of the epidermis, newborn mouse skin was incubated in ethylenediamine tetraacetic acid (EDTA) or ethylene glycol tetraacetic acid (EGTA). In EDTA, epidermolysis occurred and was confirmed by rubbing the specimen to demonstrate that a sheet of epidermis could be split off. After 30 min incubation in 0·01 mol/l EDTA the split occurred in the lower granular-upper spinous layer; after 45 min, it was in a spinous-suprabasilar location and at 60 min and later at the dermal–epidermal junction. Ultrastructurally, a clear zone of apparent intracellular oedema occurred along the cell membranes. The split then occurred intracytoplasmally through this clear zone and the adjacent cell membrane was lost.

Since incubation in EGTA at pH 7·4 did not result in epidermolysis, we suggest that the removal of magnesium rather than calcium is responsible for epidermolysis. Thirty min after the addition of either calcium or magnesium to EDTA-treated specimens, epidermolysis could no longer be demonstrated.

The phenomenon of epidermolysis is central to a knowledge of many of dermatology's most troublesome diseases, that is, the diseases in which epidermal cells separate abnormally from one another or from the dermis. Although the factors that maintain epidermal integrity are important in many skin diseases, little is known about them. Intercellular cement, desmosomes, tonofilaments, anchoring fibrils, and elastic fibres have been suggested as the elements which maintain epidermal structure even though the evidence that intercellular cement even exists in sparse; ultrastructurally desmosomes look as if they hold cells together and anchoring fibrils as if they hold epidermis to dermis.

These studies were designed to investigate the role that divalent cations play in maintaining the integrity of the epidermis and the dermal–epidermal junction.

MATERIALS AND METHODS

One- to three-day-old Webster-Swiss mice were quickly decapitated and the skin was removed from their backs with scissors. After the fatty tissue had been scraped off the dermal surface, the skin was

---

Present addresses: * Dermatologische Klinik der Universität Müchen, Frauenlobstrasse 9, D-8 München 2, Germany. † Department of Human Anatomy, School of Medicine, University of California, Davis, California 95616, U.S.A.

Reprint requests: Robert L.Dimond, M.D., Oregon Regional Primate Research Center, 505 N.W. 185th Avenue Beaverton, Oregon 97005, U.S.A.

cut into 0·5 × 0·5 cm pieces and washed in Hanks' balanced salt solution which contained calcium and magnesium (HBSS+) (Grand Island Biological Company).

These pieces were placed into beakers with the solution to be tested, and incubated at 37°C. At 15-min intervals, these specimens were removed from the solutions to determine grossly whether epidermolysis had occurred; this was done by demonstrating that a sheet of epidermis could be wiped off the specimen by rubbing a finger on it, i.e. by demonstrating the Nikolsky phenomenon.

At each time interval, traumatized (rubbed) and non-traumatized specimens were fixed in neutral buffered formalin, embedded in paraffin, sectioned at 7 μm, and stained with haematoxylin–eosin or the periodic acid-Schiff technique (PAS) for histological evaluation. For electron microscopy, tissues were fixed for 4 h in 2% paraformaldehyde plus 2·5% glutaraldehyde in 0·05 mol/l phosphate buffer, washed in 0·16 mol/l phosphate buffer (Millonig, 1962), and postfixed in 1% OsO_4 in 0·08 mol/l phosphate buffer for 2 h. (These conditions produce optimal fixation for newborn mouse skin. D.Knutson, personal communication.) Specimens were then dehydrated through graded ethanol to propylene oxide and flat embedded (Spurr, 1969). Thin sections were stained with lead citrate and uranyl acetate and viewed at 60 kV with a Philips 200 electron microscope.

The control solutions for the incubation were HBSS+ and Hanks' balanced salt solution without calcium and magnesium (HBSS−) (Grand Island Biological Company). The solutions to be tested were prepared with HBSS−, to which were added ethylenediamine tetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), or combinations of these; the pH of all solutions was adjusted to 7·4 with 7·5% sodium bicarbonate. The osmolarity of the HBSS+ and of the other solutions was 280 and 300±2 milliosmoles respectively.

TABLE 1. Effect of chelating agents and divalent cations in producing epidermolysis

Incubating solution*	Time (min)	Epidermolysis
HBSS− or HBSS+	180	−
0·04 mol/l EDTA	15	+
0·02 mol/l EDTA	30	+
0·01 mol/l EDTA	30	+ granular-spinous
0·01 mol/l EDTA	45	+ suprabasilar
0·01 mol/l EDTA	90	+ dermal–epidermal
0·005 mol/l EDTA	90	+
0·0025 mol/l EDTA	180	+
0·001 mol/l EDTA	180	−
0·02 mol/l EDTA plus 0·02 mol/l $CaCl_2$ or $MgCl_2$	180	−
0·02 mol/l EGTA (pH 7·4)	180	−
0·02 mol/l EGTA (pH 8·0)	90	+
0·02 mol/l EGTA (pH 9·0)	60	+
HBSS− or HBSS+ at pH 8·0 or 9·0	180	−
0·02 mol/l EDTA for 60 min, then add $CaCl_2$ or $MgCl_2$	30	−
0·02 mol/l EDTA for 60 min, then add HBSS−, HBSS+ or EDTA	180	+

* Unless otherwise noted all solutions were prepared in HBSS−, adjusted to pH 7·4 and incubated at 37°C.

To determine whether the loss of adhesion produced by the chelating agents could be reversed, $CaCl_2$, $MgCl_2$, or both were added to beakers which contained pieces of skin that had already been incubated in EDTA for 60 min. The molarity of the added cation solutions was the same as that of the EDTA, but twice the volume of the cation solutions was added to ensure an excess of the cation. As controls, EDTA, HBSS –, or HBSS + were added to identical specimens incubating in the EDTA.

The duration of incubation in the test solutions is shown in Table 1. Each experiment listed was performed at least five times with histological examination. On five different occasions experiments with 0·01 mol/l EDTA were evaluated with the electron microscope.

RESULTS

The results are summarized in Table 1. A positive Nikolsky sign could not be demonstrated in newborn mouse skin incubated for up to 3 h in the control solutions HBSS – or HBSS +, and light microscopic studies demonstrated no abnormalities.

A positive Nikolsky sign could be demonstrated sooner with EDTA and occurred after a shorter period with higher concentrations of EDTA (Table 1). Demonstration of the Nikolsky phenomenon produced a split in the epidermis within or just beneath the granular layer after 30 min incubation in 0·01 mol/l EDTA (Fig 1a), above the basal layer after 45 min (Fig. 1b), and at the dermal–epidermal junction above the PAS-positive basement membrane after 60 min and longer (Fig. 1c). The other concentrations of EDTA with increasing time of incubation also lowered the level of the split in the epidermis.

When newborn mouse skin was incubated in 0·02 mol/l EDTA plus an equal volume of 0·02 mol/l $MgCl_2$, $CaCl_2$, or both or in 0·02 mol/l EGTA at pH 7·4, a positive Nikolsky sign could not be demonstrated. When incubated in 0·02 mol/l EGTA at pH 8, however, a positive Nikolsky sign was demonstrated by 90 min and at pH 9 by 60 min; neither HBSS – nor HBSS + at pH 8 and 9 produced a positive Nikolsky sign by 3 h.

If excess amounts of $MgCl_2$, $CaCl_2$, or both were added to a specimen that had been incubated for 60 min in EDTA in HBSS –, a positive Nikolsky sign could no longer be demonstrated after an additional 30 min incubation, and the specimen appeared histologically normal. With comparable

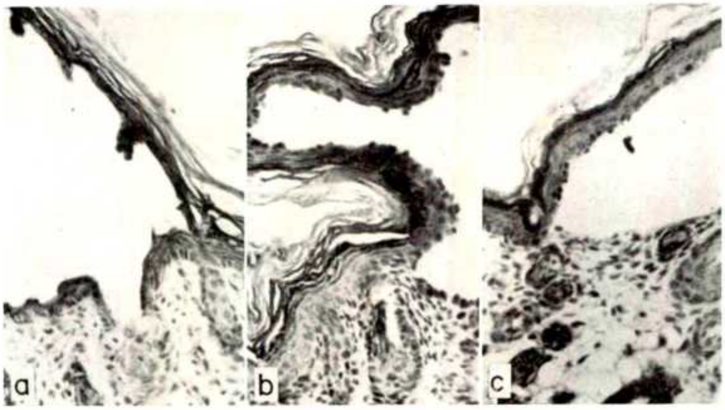


FIGURE 1. Histological appearance of newborn mouse skin after incubation in EDTA. (a) After 30 min a split was produced within the granular layer (× 200). (b) After 45 min the split was above the basal layer (× 200). (c) After 60 min the separation was at the dermal–epidermal junction (× 200).

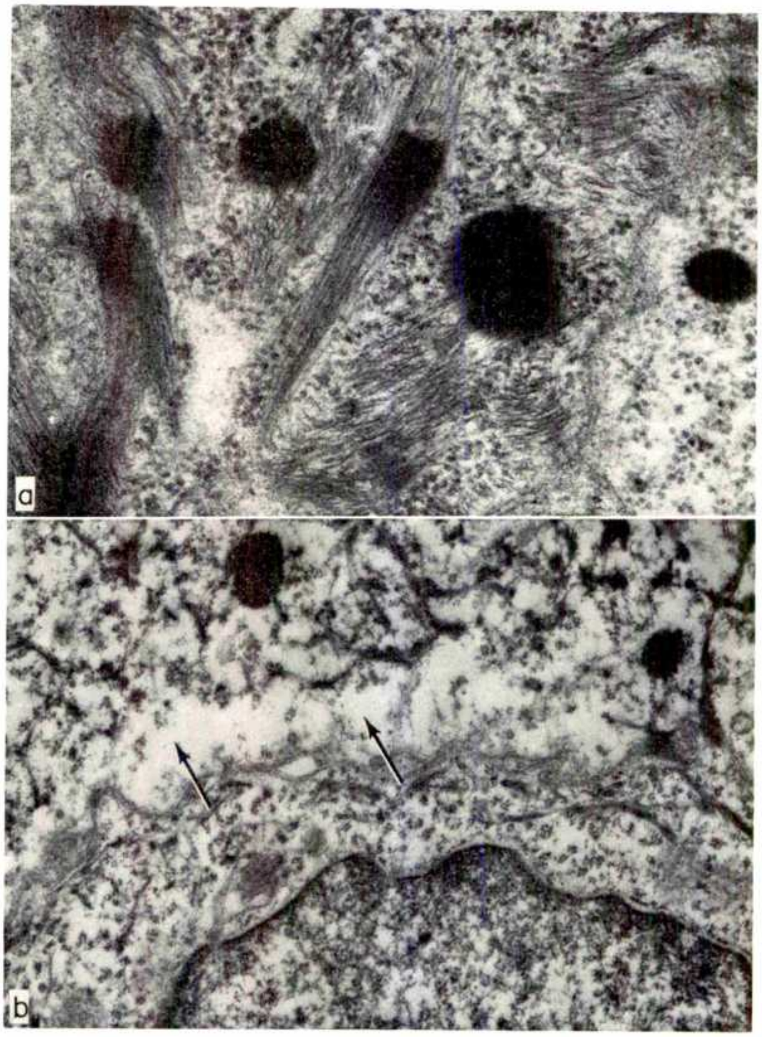


FIGURE 2. (a) The granular layer after incubation in HBSS+ for 15 min (× 77,000). (b) The granular layer after incubation in 0·1 mol/l EDTA for 15 min. Arrows indicate a clear zone in the peripheral cytoplasm (× 24,000).

specimens, to which additional EDTA, HBSS−, or HBSS+ had been added, the Nikolsky sign could always be easily demonstrated.

When newborn mouse skin was incubated for 15 min in the control solutions HBSS+ and HBSS− (Fig. 2a) no abnormalities were observed compared to tissue placed directly into the fixative and processed. But specimens incubated in 0·01 mol/l EDTA in HBSS− for 15 min showed a clear zone in the peripheral cytoplasm next to the cell membranes of the lower granular cells (Fig. 2b); no other changes were seen.

This same change was observed more extensively in specimens removed from EDTA at 30 min. After demonstration of the Nikolsky phenomenon after 30-min incubation, a split occurred in the lower granular layer usually at the level where spinous cells change to keratohylin-containing cells (Fig. 3). This separation passed through the cytoplasm at the level of the peripheral clear zone beneath

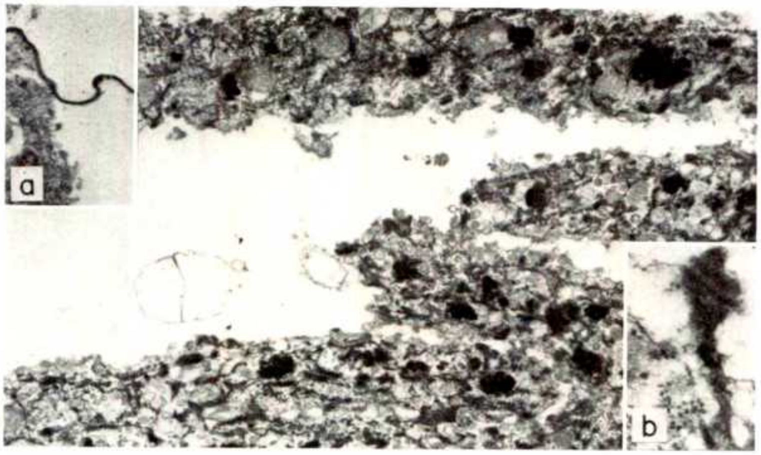


FIGURE 3. Skin incubated in EDTA for 30 min. Demonstration of the Nikolsky phenomenon produced a separation through the granular layer (× 9400). Insert (a). Fragments of a cell membrane seen in the area between the split (× 11,000). Insert (b). A desmosome in the granular layer attached by tonofilaments (× 58,000).

the membrane and caused fragments of membranes, mitochondria, and other cellular contents to be extruded from the cells. The membrane could be seen peeling off an occasional cell (Fig. 3, insert a), or the attachment plaque of a desmosome still held by tonofilaments could be seen extending out of the cytoplasm (Fig. 3, insert b). In the upper granular layer and stratum corneum above the split, the cells appeared normal. Desmosomes and tonofilaments were intact and there was no apparent change in the intra- or extracellular keratinosomes. We did note that in both treated and control specimens there was an apparent lack of these organelles in some areas and an unusually large number in others. In both control and treated specimens a split occurred between cells with intact membranes only in the stratum corneum or between the stratum corneum and the granular layers; here the normally thickened cell membranes did not change after exposure to EDTA. At this time also a clear zone could often be seen in the cytoplasm of basal layer cells along both the superior and inferior surfaces of EDTA-treated specimens. In addition, the intercellular space between spinous cells had widened with apparent intercellular oedema and with or without microvilli formation (Fig. 4); this same finding observed in control specimens at this time was considerably less extensive.

After 45-min incubation in EDTA, the specimens showed less clearing of the peripheral cytoplasm

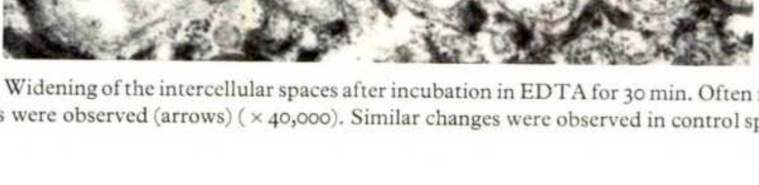


FIGURE 4. Widening of the intercellular spaces after incubation in EDTA for 30 min. Often microvilli formations were observed (arrows) (× 40,000). Similar changes were observed in control specimens.

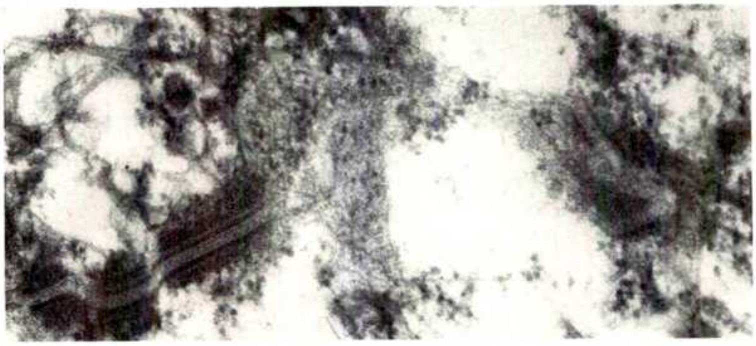


FIGURE 5. Desmosomes appear normal but cell membranes are not well visualized after incubation in EDTA for 45 min (× 69,000).

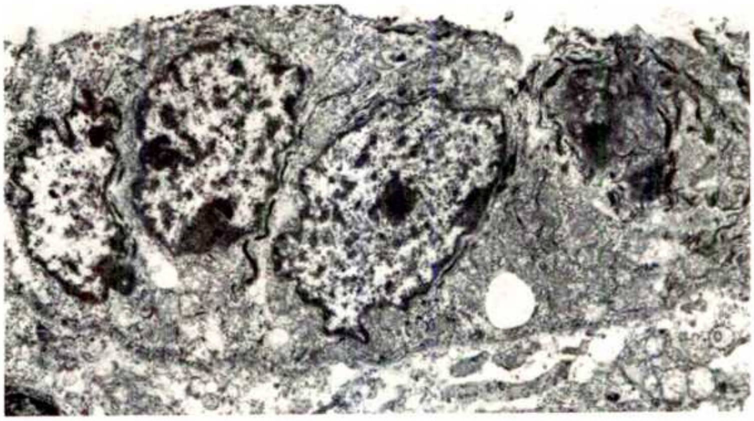


FIGURE 6. Suprabasilar split after incubation in EDTA for 45 min and application of mild trauma (× 6000).

in the spinous and granular layers. Instead the membranes of these cells could not be visualized although desmosomes were still present and unchanged in appearance (Fig. 5). The clear peripheral zone in the basal layer was more apparent than before. Those specimens to which mild trauma had been applied showed a split generally in a suprabasilar location but also through the clear zone of the peripheral cytoplasm at the superior aspect of the basilar cells (Fig. 6). Despite some intercellular oedema between the basal cells, the desmosomes were intact. At this time, a few cells throughout the tissue were markedly oedematous and the mitochondria were swollen, had lost their internal structure, and occasionally appeared only as membranous ghosts. The clear peripheral zone along the inferior plasma membrane of the basal cells was more marked, and the upper dermis also showed some widening of the spaces between collagen fibres.

Specimens that had been incubated for 60 min in EDTA now had more marked intercellular oedema just above the basal plasma membrane (Fig. 7a). Tonofilaments on the attachment plaques on the plasma membrane had wide spaces between them. Demonstration of the Nikolsky phenomenon at this time produced a split through the lower part of the basal cells. As a result, either the basal plasma membrane with its hemidesmosomes remained attached to the dermis (Fig. 8a) or the entire basal plasma membrane was torn from the dermis and seen only as fragments in the split (Fig. 8b). In most areas no plasma membrane remained on the basal cell. The upper epidermis resembled the

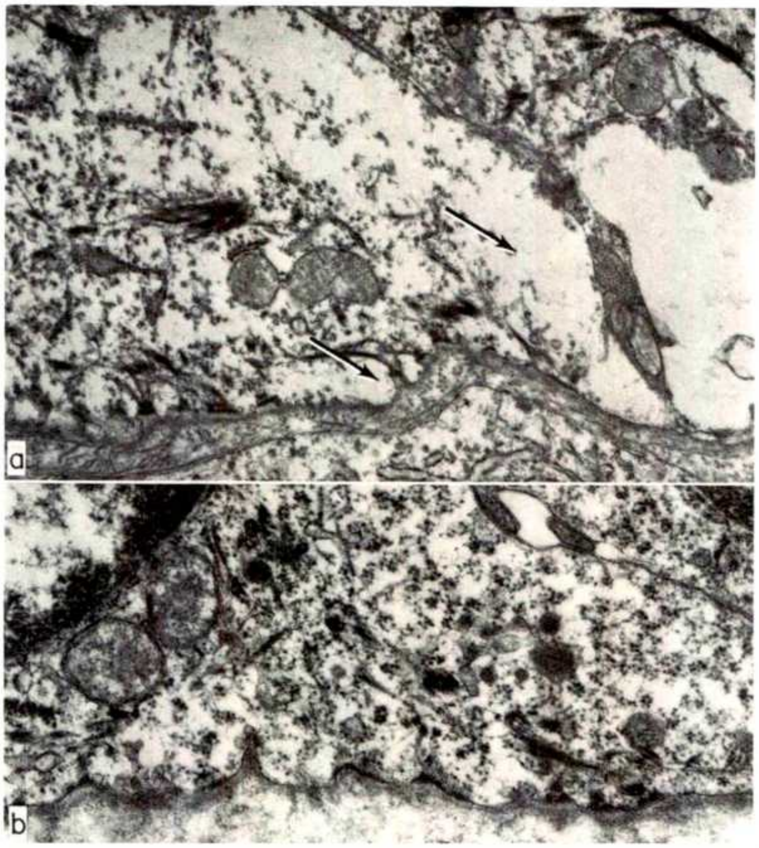


FIGURE 7. (a) Intracellular oedema (arrows) above the basal plasma membrane after incubation in EDTA for 60 min (× 69,000). (b) Control specimen incubated in HBSS+ for 60 min shows no peripheral clear zone around membranes (× 35,000).

specimens incubated for 45 min. In controls incubated in HBSS+ for 60 min, we observed occasional areas of an intracellular clear zone near the cell membranes, a widening of intercellular spaces, and separation of collagen fibres in the upper dermis although most areas appeared normal (Fig. 7b). As in the treated specimens, a few degenerating cells were seen.

By 90 min these changes in the control specimens were more obvious although the tissue still looked mostly normal. Specimens incubated in EDTA had a split at the dermo-epidermal junction. Unlike the findings at 60 min, when the split might leave the inferior plasma membrane with the dermis, now the inferior plasma membrane was always lost and only fragments of membranes could be seen in the plane of the split. The inferior basal cell had no visible membrane although attachment plaques could be seen. More separation of collagen fibres was observed in the upper dermis and more degenerating cells in the epidermis. At 120 min, the split remained in the same level and degenerative changes were more apparent in the cells. Control specimens also had some cells with degenerative changes as well as intracellular and intercellular oedema.

Specimens that had been incubated for 60 min in 0·01 mol/l EDTA and for 30 min more in $CaCl_2$ or $MgCl_2$ no longer showed a positive Nikolsky phenomenon. Ultrastructurally, however, considerable change persisted: clearing of the peripheral cytoplasm, the poorly visualized membranes, and degenerative changes in the cellular contents (Fig. 9).

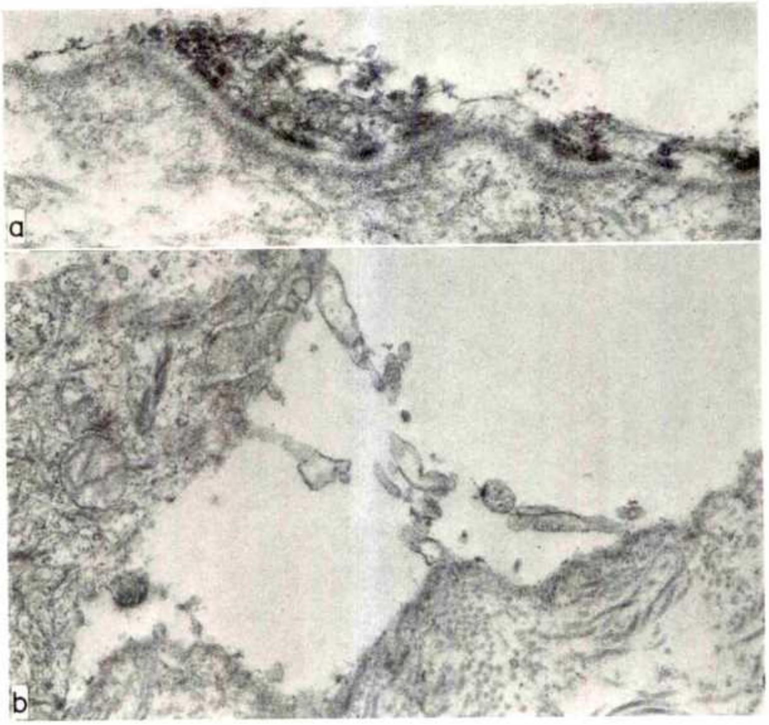


FIGURE 8. (a) Dermis after incubation in EDTA for 60 min and the demonstration of the Nikolsky phenomenon. The plasma membrane and its hemidesmosomes remain attached to the dermal side of the split (× 58,000). (b) In this specimen, also incubated for 60 min, only the basal lamina remains with the dermis, and the plasma membrane of the basal cell is seen only as fragments (× 25,000).

DISCUSSION

The first important result of these experiments is the demonstration that divalent cations play a critical role in maintaining skin structure not only between dermis and epidermis but also with the epidermis itself. The result of chelating divalent cations in skin by means of EDTA is epidermolysis; thus we conclude that divalent cations play an important role in maintaining epidermal integrity and preventing epidermolysis.

Ringer (1890) was probably the first to show that divalent cations are necessary for intercellular adhesion. Calcium- and magnesium-free solutions with and without added EDTA are standard tissue-culture solutions used to separate cells and tissues (reviewed by Mascona, 1962). The first to use EDTA experimentally on skin were the embryologists who used it to separate chick embryo epidermis for transplantation experiments (Bell *et al.*, 1962). Methods of using EDTA to separate epidermal cells from one another in order to obtain cells from the various layers of the epidermis have been described (Gumucio, Feldkamp & Bernstein, 1967; Stern & Sekeri-Pataryas, 1972). Thus evidence that divalent cations are required to maintain the structural integrity of skin is not new.

The second important point suggested by these studies is that magnesium rather than calcium is the cation needed to maintain adhesion. Previous studies (reviewed by Mascona, 1962) of the cellular adhesiveness effected by EDTA had assumed that calcium was the critical element and ignored the fact that magnesium is also bound almost as well by EDTA. EGTA also binds magnesium but much more weakly than calcium (Schmid & Reilley, 1957). An effect produced in biological systems by EDTA but not by EGTA is considered to be due to magnesium whereas an effect produced by both

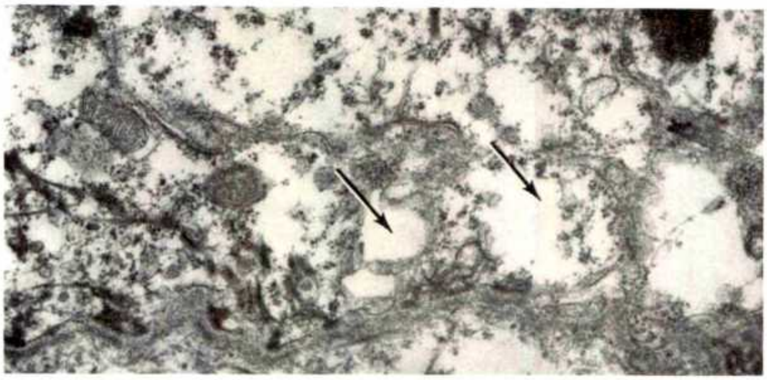


FIGURE 9. Although a positive Nikolsky phenomenon could not be demonstrated, clearing of the peripheral cytoplasm persists (arrows) after incubation for 60 min in EDTA and 40 min in $MgCl_2$ (× 34,000).

EDTA and EGTA is believed to be produced by calcium. The fact that EGTA failed to produce epidermolysis and EDTA did is evidence that in the normal epidermis magnesium is important for intra-epidermal and dermal–epidermal integrity. EGTA binds calcium in biological systems at pH 7·4 and above (Millington, Critchley & Tovell, 1966). Why it should produce the loss of adhesion at pH 8 and pH 9 but not at pH 7·4 is not clear, but the fact that it does may indicate that calcium is actually the critical factor. Scaletta & MacCallum (1972) reported similar findings in human oral mucosa. It should also be noted that since EDTA chelates other cations besides magnesium and calcium, the mechanism of epidermolysis could be related to one of the trace metals.

The third important finding of this study is that the loss of adhesion produced by EDTA can be reversed by adding magnesium or calcium to the system. It is remarkable that cells in such a well-differentiated tissue as skin can lose their adhesion to one another and then quickly regain it. This reversal of epidermolysis has not been described in skin before but was reported by Scaletta and MacCallum in human oral mucosa (1972). The fact that calcium terminates epidermolysis when these experiments indicate that the removal of magnesium causes epidermolysis is not surprising since in many biological systems magnesium and calcium can be temporarily substituted for each other.

Our electron microscopic studies show that the first ultrastructural change produced by EDTA is a clearing of the peripheral cytoplasm of epidermal cells followed by more general oedema of the cell and loss of cell membrane when mild trauma is applied to the tissue. The split occurs at different levels of the epidermis, depending on when the trauma is applied to the tissue. Higher level cells seem to be more sensitive to the effects of EDTA than lower cells. First a granular layer split occurs, then a spinous and suprabasilar split, and lastly a dermal–epidermal split; the split, however, is never below the basal lamina. In some areas, the intercellular space widens with microvillous formation, but since this also occurred in some control specimens, it may not be significant. Scaletta & MacCallum (1972), who treated human oral mucosa with 0·02 mol/l EDTA, reported that after 60 min a dermal–epidermal separation occurred above the basal lamina. They did not describe the peripheral cytoplasmic clearing observed by us in newborn mouse skin but did not examine their tissue before separating the dermis from the epidermis. They did observe whorls of membrane-like material beneath the separated basal cells, and their published pictures show a widespread loss of plasma membranes on the basal part of basal cells. Therefore, we believe that their results, if not their interpretations, are compatible with ours. They concluded that the removal of divalent cations causes a change in the hemidesmosomes that unite the epidermis to the lamina propria. We, however, believe that the effect of EDTA is more likely a disturbance of some membrane or perhaps intracytoplasmic function which leads to oedema of the peripheral cytoplasm and weakening of the membrane.

In a similar study on newborn rat epidermis treated with trypsin, Fukuyama, Black & Epstein (1974) found that the basal lamina as well as the interdesmosomal material disappears. Scaletta & MacCallum (1974) have reported similar findings in the human oral mucosa treated with trypsin. But in their studies with EDTA as in ours neither basal lamina nor interdesmosomal material disappeared; therefore EDTA clearly causes the separation in a different manner than trypsin.

Borysenko & Revel (1973) have also shown that the desmosomes of several stratified squamous epithelia (rat oesophagus, frog skin, and human oral mucosa) are not altered by treatment with EDTA. However, when non-squamous epithelia such as rat and frog gut and liver were treated, the intermediate dense band of desmosomes was lost, as Fukuyama *et al.* (1974) observed in newborn rat skin treated with trypsin. Thus desmosomes of different tissues must be chemically different although morphologically similar.

Since our studies show changes that also occur in various pathological conditions in which epidermolysis or acantholysis occur, we suggest that the mechanism of epidermolysis in these diseases may involve the same pathways as the epidermolysis produced by EDTA.

ACKNOWLEDGMENTS

The authors are indebted to Joyce Beeman, Kunie Mah, and Yvette Frutiger for skilful technical assistance.

This study was supported by a grant from the Dermatology Foundation and USPHS Grants RR 00163, AM 05512, AM 5300, and AM 40086-04, from the National Institutes of Health and is Publication No. 817 from the Oregon Regional Primate Research Center.

REFERENCES

Bell, E., Gasseling, J.W., Saunders, J.W. & Zwilling, E. (1962) On the role of ectoderm in limb development. *Developmental Biology*, **4**, 177.

Borysenko, J.Z. & Revel, J.P. (1973) Experimental manipulation of desmosome structure. *American Journal of Anatomy*, **137**, 403.

Fukuyama, K., Black, M.M. & Epstein, W. (1974) Ultrastructural studies of newborn rat epidermis after trypsinization. *Journal of Ultrastructure Research*, **46**, 219.

Gumucio, J., Feldkamp, C. & Bernstein, I.A. (1967) Studies on localization of 'histidine-rich' peptide material present in epidermis of the newborn rat. *Journal of Investigative Dermatology*, **49**, 545.

Mascona, A.A. (1962) Cellular interactions in experimental histogenesis. *International Review of Experimental Pathology*, **1**, 371.

Millington, P.F., Critchley, D.R. & Tovell, P.W.A. (1966) The role of calcium in the isolation of brush borders from epithelial cells of rat small intestine. *Journal of Cell Science*, **1**, 415.

Millonig, G. (1962) Further observations on a phosphate buffer for osmium solutions in fixation. In: *Fifth International Congress of Electron Microscopy* (Ed. by S.S.Breese, Jr). Academic Press, New York.

Ringer, S. (1890) Concerning experiments to test the influence of lime, sodium, and potassium salts on the development of ova and the growth of tadpoles. *Journal of Physiology (London)*, **11**, 79.

Scaletta, L.J. & MacCallum, D.K. (1972) A fine structural study of divalent-cation-mediated epithelial union with connective tissue in human oral mucosa. *American Journal of Anatomy*, **133**, 431.

Scaletta, L.J. & MacCallum, D.K. (1974) A fine structural study of human oral epithelium separated from the lamina propria by enzymatic action. *American Journal of Anatomy*, **140**, 383.

Schmid, R.W. & Reilley, C.N. (1957) New complexon for titration of calcium in the presence of magnesium. *Analytical Chemistry*, **29**, 264.

Spurr, A.R. (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research*, **26**, 31.

Stern, I.E. & Sekeri-Pataryas, K.H. (1972) The uptake of ^{14}C-leucine and ^{14}C-histidine by cell suspensions of isolated strata of neonatal rat epidermis. *Journal of Investigative Dermatology*, **59**, 264.

British Journal of Dermatology (1976) **95**, 35.

Necropsy and ultrastructural findings in histiocytic medullary reticulosis

J.PIÑOL-AGUADÉ, J.FERRANDO, J.M.TOMÁS, C.MIERAS
AND J.PEYRÍ

Departments of Dermatology and Pathology, Faculty of Medicine, University of Barcelona, Spain

Accepted for publication 7 November 1975

SUMMARY

In a case of histiocytic medullary reticulosis with protracted course and necrotic skin lesions, histological study revealed infiltration of the fat tissue by atypical reticulohistiocytic cells, areas of associated fat necrosis and vascular lesions. Autophagocytosis (phagocytosis of erythrocytes, erythroblasts, platelets, lymphoid cells, and nuclear debris) was found in biopsy specimens of the subcutaneous infiltrates, lymph nodes, and bone marrow, and in necropsy specimens of skin, iliac mesenteric lymph nodes, bone marrow, liver, spleen, and suprarenal glands.

Electron microscopy of various viscera showed abnormal histiocytic cells containing multiple phagocytosed erythrocytes and other blood cells in different stages of destruction and digestion. Multi-membranous bodies also were present. Defective lysosomal degradation of intracytoplasmatic ceroids in this condition is postulated.

In a previous paper (Piñol-Aguadé *et al.*, 1974) we described the clinical and histological findings in three cases of non-tumoural malignant reticulosis with necrotic lesions of the skin. The general characteristics of these cases closely resembled those of the entity described by Scott & Robb-Smith in 1939 under the name of 'histiocytic medullary reticulosis' (HMR), and the similarity was confirmed by necropsy studies in two of our cases.

Here we give detailed necropsy findings in the third case, together with an account of the ultrastructure of lesions in different viscera. We believe the ultrastructural findings to be of particular interest because the necropsy was performed only 2 h after death. We are aware of no other electron microscopical examination of visceral tissues in this disease. However, Zawadzki, Peña & Fisher in 1969 described the ultrastructural findings in bone-marrow smears. Recently Warnke, Kim & Dorfman (1975) have published the necropsy findings in fourteen cases but ultrastructural data have not yet been reported.

Correspondence address: Dr J.Piñol Aguadé, Department of Dermatology of University of Barcelona, c/ Casanovas 143, Barcelona, Spain.

CASE REPORT

The clinical and laboratory data relating to this case have been published previously (Piñol-Aguadé *et al.*, 1974). Briefly, the patient was a 30-year-old woman who had suffered for 4 years from bouts of fever, general malaise, asthenia, anorexia, and loss of weight. There were concomitant outbreaks of subcutaneous nodules, mostly in the lower extremities, which showed a tendency to undergo necrosis and ulceration.

The bouts alternated with asymptomatic intervals during which the patient could lead a practically normal life. The final outbreak of cutaneous lesions was accompanied by persistent high fever, intense abdominal pain, and jaundice. Petechiae and ecchymoses appeared, and the patient died.

Laboratory investigations revealed persistent and progressive leukopenia, with hypochromic anaemia and thrombocytopenia in the terminal phases. Marrow aspiration and biopsy of the iliac crest showed an increase of reticulo-endothelial elements and autocytophagic phenomena.

Histological examination of the ulcers showed a lymphohistiocytic infiltration around the blood-vessels of the deep dermis. Some histiocytes contained a large nucleus with multiple and irregular nucleoli. But the characteristic histological pattern consisted basically of panniculitis, in which areas of lipogranuloma alternated with others infiltrated with atypical reticular elements, some of them showing erythrophagocytosis. Some of the reticulo-histiocytic elements showed a Sternberg-like appearance, and most of them had an eosinophilic cytoplasm. Mitoses were abundant. Some of the vessels were thrombosed and others evidenced fibrinoid necrosis.

Autopsy findings

Gross. The pleural cavity contained a small quantity of fluid (50 cc). The pericardium had no macroscopic external lesions. The liver was pale with a smooth surface free of nodules. The spleen was large (400 g) and much congested but had no macroscopic nodules. The kidneys had a smooth surface, but petechiae around the calyces and renal pelvis were found. The suprarenal glands were atrophic (total thickness 2 mm). The mesenteric lymph nodes were of normal size but appeared congested on cross section. The lymph nodes of the left iliac chain were enlarged. The bone marrow seemed to be congested and a yellowish white nodule of 10 mm diameter was observed in it. There was an extensive haematoma in the left psoas muscle. The remaining viscera showed no gross abnormality.

Histology. Microscopic examination of the spleen disclosed massive infiltration by rounded or polygonal reticular cells with globular irregular nuclei and abundant pasty eosinophilic cytoplasm. Many of the cells contained erythrocytes (Fig. 1), with varying degrees of autophagocytosis. Some of the phagocytes contained a single erythrocyte and others many, grouped to give a sausage-like appearance. Other cells had rounded vacuoles in their cytoplasm with outlines resembling those of the intracytoplasmic red cells. The splenic structure was greatly altered, and the white pulp was more easily distinguishable than the red.

The histological structure of the liver was intact, though its sinusoids were somewhat congested and contained occasional histiocytes showing erythrophagocytosis (Fig. 2).

In one mesenteric lymph node, and especially in a node of the iliac chain, massive cellular invasion similar to that in the spleen was observed. The histiocytic cells with malignant appearance were less differentiated than in other organs and formed a compact focus.

The sternal bone marrow showed various cell forms, including some erythrophagocytic histiocytes. A small focus of undifferentiated cells was seen, which was similar to those of the lymph nodes and spleen.

The suprarenal glands manifested severe lipid depletion and considerable infiltration of erythrophagocytic histiocytes (Fig. 3) with vacuoles, like those seen in the haematopoietic organs.

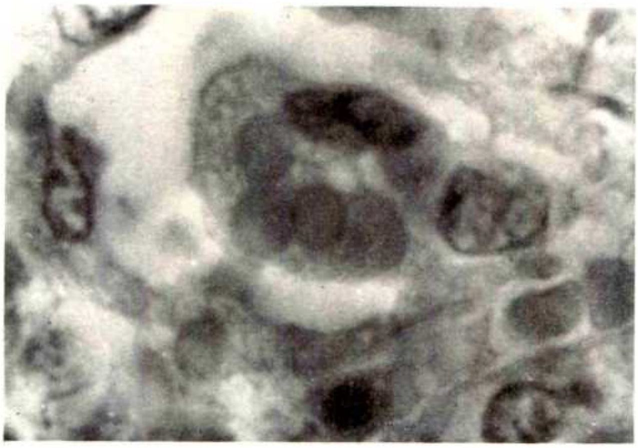


FIGURE 1. Spleen: voluminous histiocyte with four phagocytosed erythrocytes (H & E, × 1500).

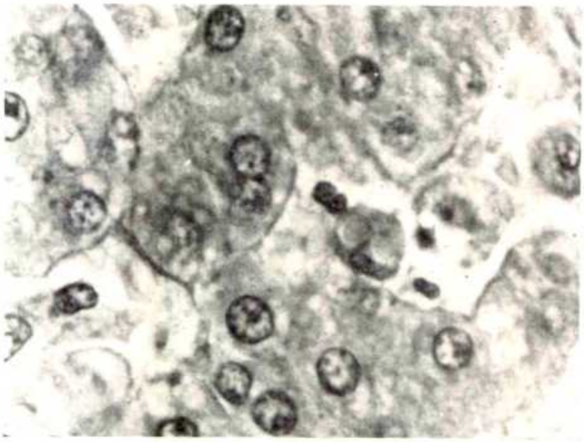


FIGURE 2. Liver: erythrophagocytosis in sinusoids (H & E, × 600).

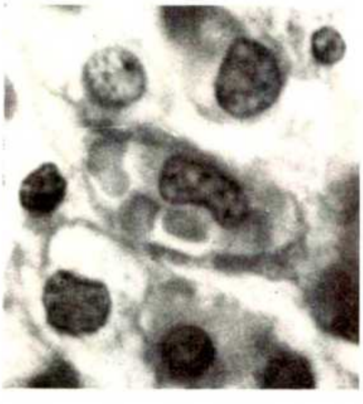


FIGURE 3. Suprarenals: infiltration by erythrophagocytic histiocytic cells (H & E, × 1000).

In the renal tubules, hyaline casts were abundant. Some proximal tubules had suffered degenerative cell changes, but the glomeruli were normal. No infiltration was found.

The lungs showed slight emphysema but no infiltration.

Electron microscopic studies

Materials and methods. Autopsy specimens were taken from the spleen, liver, sternal bone marrow, iliac and mesenteric lymph nodes, and skin of the tibial area taken 5 mm from an ulcerated lesion. The specimens were pre-fixed in a solution of glutaraldehyde 2·5% in Sörensen's phosphate buffer (pH 7·4) (Sörensen, 1963), for 2 h. They were then fragmented and post-fixed in Dalton's solution (osmium tetroxide) for 2 h. After dehydration in acetone and uranyl acetate, the specimens were embedded in epoxy resin (Araldite). Sections (0·5–1 μm) were cut and stained with methylene blue (Richardson, Jarett & Finke, 1960). Thin sections were cut on a Reichert OmU3 microtome, contrasted with Reynolds' lead citrate solution (Reynolds, 1963), and examined with a Hitachi Hu-12 electron microscope with an accelerating voltage of 75 kV.

Results. The terminal process and the 2-h period of autolysis may have been the cause of some structural modifications such as dilatation of the canalicular structure within cells and ballooning and cristolysis of the mitochondria.

The specimens of skin, iliac lymph node, and spleen showed invasion by large histiocytic cells, obliterating the normal structure of the tissue. The skin sample showed severe tissue damage resulting from its location adjacent to the necrotic tissue of the ulcer.

Elements associated with granulation tissue were identified: There were large lipophagic and macrophagic cells with intracytoplasmic inclusions whose appearance suggested erythrophagocytosis. In the samples of mesenteric lymph nodes and bone marrow, no important abnormalities were found.

The liver cells showed severe autolytic phenomena. Many of the mitochondria contained paracrystalline structures that eventually filled the greater part of the matrix. There were some giant mitochondria. Haemosiderin granules and residual bodies with or without ferritin particles were abundant. The Kupffer cells contained many residual bodies but showed no erythrophagocytosis. There were no apparent foci of haematopoiesis.

The specimens of iliac lymph node and spleen showed extensive infiltration with many instances of erythrophagocytosis, particularly in the spleen sample. Some macrophages contained as many as eight apparently intact red cells in their cytoplasm (Fig. 4).

The abnormal histiocytes (30–40 μm) had large eccentric nuclei with an indented outline and heterochromatin heavily concentrated at the periphery. In some cells, scattered chromocentres were found. There were some nucleoli with nucleolonema. The cytoplasm was voluminous, with occasional pseudopodal processes of varied size and shape—sometimes adjacent to erythrocytes, suggesting the initial stages of erythrophagocytosis. Pinocytotic vesicles were frequent. Mitochondria were fairly abundant, small, and generally oval or irregular in shape. The Golgi apparatus was well developed, consisting of dilated cisterns near which centriolar structures were seen in some cells. The rough endoplasmic reticulum was well developed and dilated. There were many polyribosomes. The agranular endoplasmic reticulum was dilated. There were also vacuoles and vesicles of different sizes.

Some cells contained tortuous microtubules. Fine cytoplasmic filaments (10 μm in length) were common, either scattered haphazardly or grouped in bundles.

Other observations included frequent evidence of lipids, secondary lysosomes, and multi-membranous bodies (Fig. 5).

In many cells there was an exceptionally large number of small or medium-sized bodies enclosed

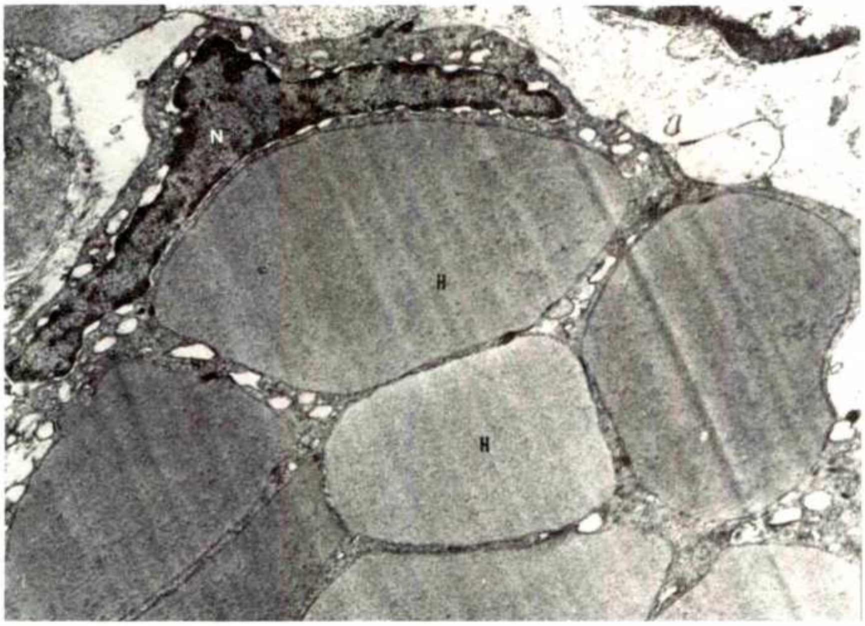


FIGURE 4. Part of a large macrophagic spleen cell, with nucleus (N) displaced to periphery and deformed by sausage-like formation of phagocytosed red cells (H) (× 6000).

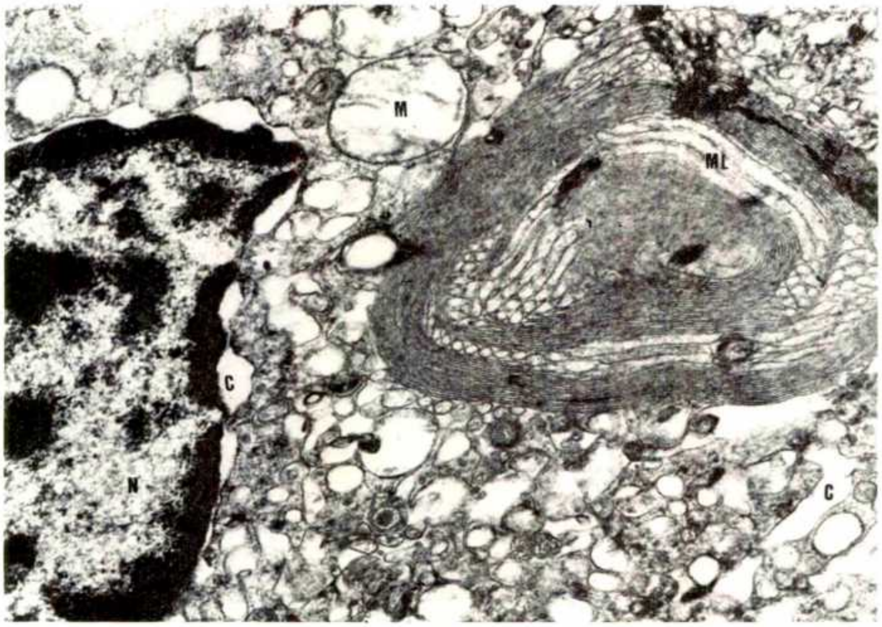


FIGURE 5. Spleen cell. N = irregular nucleus with heavy deposits of chromatin. M = mitochondria. ML = multi-laminar (or multi-membranous) body. C = dilatation of canalicular structures of the cell (× 10,000).

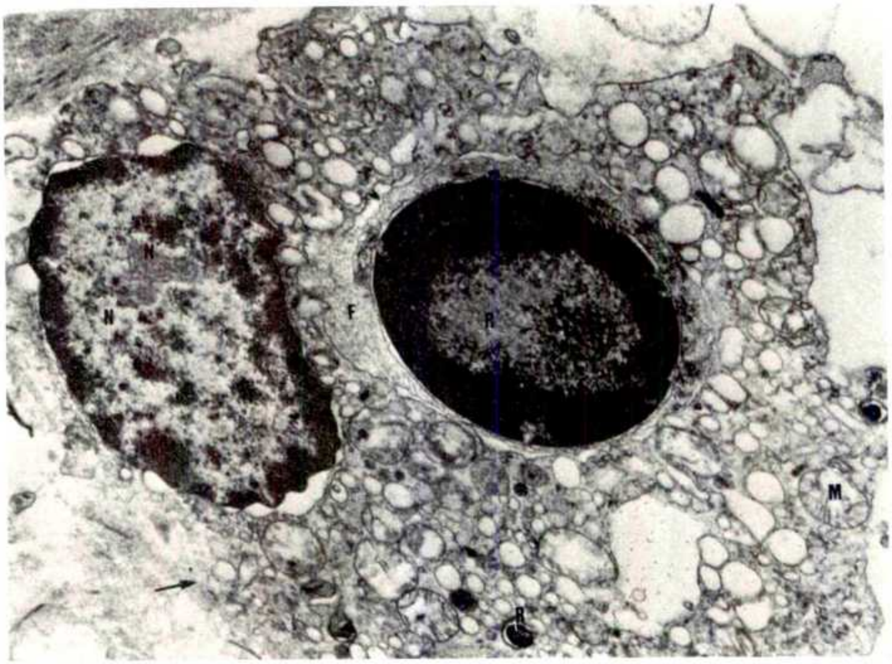


FIGURE 6. Infiltrating macrophage in iliac lymph node. R′ = nucleated element of red-cell series in large vacuole of macrophage. The plasma membrane is ruptured and cytoplasmic contents retracted in form of ferritin (F); N = nucleus; M = mitochondria; R = residual body; N′ = nucleoid; Arrow, ruptured plasma membrane (× 6000).

in a single or double membrane. Usually the contents were dense; occasionally there was a dense core surrounded by a clear or lucid halo. These bodies often contained ferritin or formed part of autophagic vacuoles with cytoplasmic debris.

Most of the phagocytosed cells were mature erythrocytes (Fig. 4), but phagocytosis of reticulocytes, erythroblast (Fig. 6), platelets (Fig. 7), and debris of other nucleated cells also were seen.

Red cells in some localities showed various stages of degradation (Fig. 7). The sequence was indicated by progressive condensation of the matrix, rupture of the membrane, and extraction of iron in the form of ferritin. In other areas, the majority of intracytoplasmic red cells showed a similar stage of degradation. This was probably because the samples were taken during the acute terminal phase of erythrophagocytosis or, as some authors suggested (Gemsa, Fudenberg & Schmid, 1974), resulted from the heavy doses of corticosteroids administered in the last days of the patient's life.

COMMENT

The notable progress in immunology during the last decade has made possible a functional classification of the lymphoreticular system. Today, lymphomas are labelled according to whether their components are thymus-derived (T cells), bursa-equivalent (B cells), or histiocytic. Working along these lines, Lukes & Collins (1974) devised the following classification of lymphomas: (1) with undefined cells, (2) with T cells, (3) with B cells, (4) with histiocytes, and (5) unclassifiable. Even within this primordial division, it is possible to make subdivisions according to the presence or absence of membrane markers or the secretion of immunologically active substances (Salmon & Seligmann, 1974).

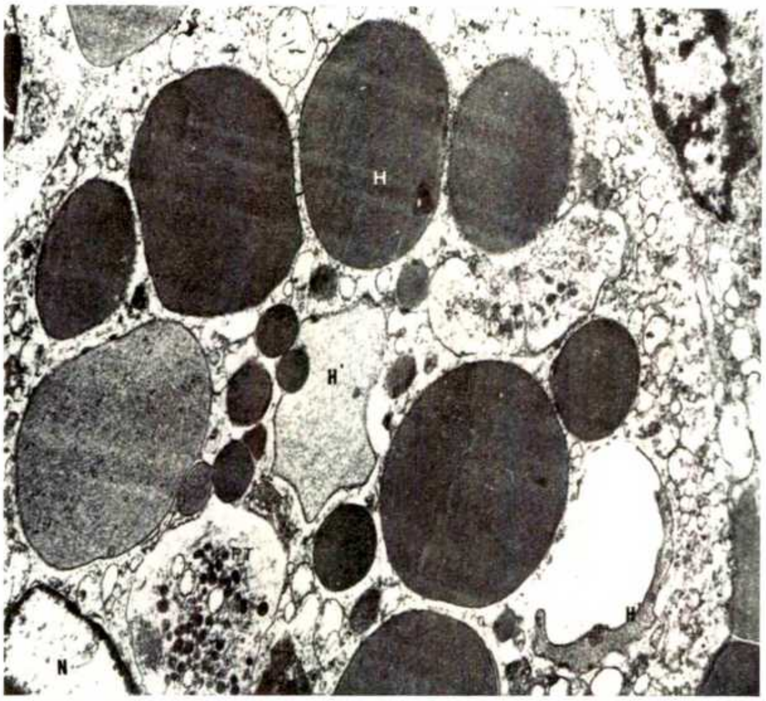


FIGURE 7. Part of a spleen cell with cytoplasm full of erythrocytic forms (H). H′ = red cells in advanced stage of degradation (Simon & Burke, 1970). N = nucleus; PT = platelets (× 5000).

HMR may be considered the most representative example of the fourth Lukes-Collins group. There is evidence to support its inclusion in this group rather than among the cases so far defined as reticulosarcoma or reticulohistiocytic lymphoma. Stein, Lennert & Parwarech (1972) demonstrated that a large number of these lymphomas are really proliferations of B cells that appear pseudoreticular or histiocytic as a result of transformation of the lymphocytes.

The ultrastructural features described in this paper (abundance of lysosomes, multi-membranous bodies, and pseudopodal processes) and the macrophage activity evident from histological study of this case justify its inclusion within the group of lymphoreticular lymphoma with a histiocytic function.

It has been argued that HMR should be considered a reactive process and not be classified with lymphomas (i.e. true neoplasms), since it is systemic in character and does not form tumoural proliferations. However, there are good reasons against this opinion. For example, some authors (Lukes & Collins, 1974) maintain that the Reed-Sternberg cells represent the polyploid aspect of the immunoblasts during transformation of the lymphocytes rather than being transformed malignant cells, and that this transformation may develop from multiple causes (among them certain viral infections and infectious mononucleosis). In this way other lymphomas could occur as reactive phenomena. The dividing line between reaction and neoplasm is less clear now than it was some years ago.

Among histiocytic lymphomas, erythrophagocytosis may be seen in histiocytosis X as well as in HMR, but the latter does not have the rod-shaped or racket-shaped granules seen in histiocytosis X (Breathnach, 1971). The question arises: are these distinct morphological structures found in the two diseases only different stages of functional differentiation of the histiocyte? In HMR there would be an increase of the phagocytic capacity, and in histiocytosis X an increase of the metabolizing potential. In this context it is worth mentioning that all the ultrastructural cytological formations observed in the present case (pseudopodal processes, autophagocytosis phenomena, multimembranous bodies, and highly developed Golgi apparatus) represent different aspects of the phagocytic capability of the histiocyte.

In the case reported by Zawadzki *et al.* (1969), these same cytoplasmic characteristics were observed in the bone marrow. And in their case the nuclear chromatin was more diffuse than in ours.

The multi-membranous or multi-laminar figures are of particular interest. In spite of the fact that similar laminated profiles are known to appear during cell lysis, we postulate that they represent true pathological change. Similar images have been found recently in reticulohistiocytoma (Hashimoto & Pritzker, 1973; Laugier *et al.*, 1975), in amiodarone pigmentation (Olmos & Laugier, 1974) and in the sea-blue histiocyte syndrome (Sawitsky, Hyman & Hyman, 1954; Silverstein, Ellefson & Ahern, 1970; Reynès, Kalifat & Diebold, 1973). This last name has been applied to idiopathic lipidosis of the liver and spleen with an excess of ceroids and increased mucopolysaccharides in the urine. In these cases the May–Grünwald–Giemsa stain reveals histiocytes with blue granules in the cytoplasm. These could be the multi-laminar formations observed with the electron microscope in splenic red pulp (Reynès *et al.*, 1973).

Other authors (Howes, Price & Blumberg, 1964; Biava, 1965; Györkey, Shimamura & O'Neal, 1967; Ferrans *et al.*, 1971; Rywlin *et al.*, 1971a) considered these concentric fingerprint-like laminar formations to be characteristic of the ceroids.

The excess of ceroids that gives rise to sea-blue histiocytes has been observed in widely differing diseases, for example in conditions with massive cell destruction, chronic myeloid leukaemia (Sundberg *et al.*, 1964; Kattlove *et al.*, 1969; Lee & Ellis, 1970; Dosik, Rosner & Sawitsky, 1972), thrombocytopenia (Landing *et al.*, 1961; Saltzstein, 1961; Hill, Speer & Gedrikoglu, 1963; Dollberg *et al.*, 1965; Quinton, Wurzel & Czernobilsky, 1967; Couderc *et al.*, 1969; Cazal *et al.*, 1971; Quattrin *et al.*, 1972), hyperlipoproteinaemia of type I (Ferrans *et al.*, 1971) or type V (Rywlin *et al.*, 1971b), dyslipidosis (Terry, Sperry & Prodoff, 1954; Kristenson & Sourander, 1966; Lowden, Barson & Wentworth, 1970), albinism with hypocholesterolaemia (Maranoff, Fite and Frumin, 1972) and lecithin cholesterol acyltransferase deficiency (Jacobsen, Gjone & Hovig, 1972). This feature has been found also in cases of chronic granulomatous disease (Ford *et al.*, 1962; Bartman, van de Velde & Friedman, 1967) and of rupture of the spleen (Winkler *et al.*, 1969) and in siblings with splenomegaly and neurological disorders (Jones *et al.*, 1970; Lake, Stephans and Stephans, 1970).

These multi-membranous bodies may be partly metabolized lysosomal products of the excess of ceroids in the cytoplasm. The presence of multi-laminar formations in this particular case can be explained by the abundance of phagocytosed cellular debris. Most of the latter consists of lipids, which are important constituents of cellular membranes. This would lead to the same lysosomal defect of degradation that is found in the sea-blue histiocyte syndrome.

REFERENCES

BARTMAN, J., VELDE, R.L. VAN DE & FRIEDMAN, F. (1967) Pigmented lipid histiocytosis and susceptibility to infection: ultrastructure of splenic histiocytes. *Pediatrics*, **40**, 1000.

BIAVA, C. (1965) Electron microscopic studies on periodic acid-Schiff-positive non glycogenic structure in human liver cells. *American Journal of Pathology*, **46**, 435.

BREATHNACH, A.S. (1971) Dermis, connective tissue and individual cells. In: *An Atlas of the Ultrastructure of Human Skin*, 1st edn, p. 178. Churchill, London.

CAZAL, P., KARN, P., EMBERGER, J.M., NAVARRO, M. & RIZKALLA, N.A. (1971) Splénomégalie à céroïdes (syndrome des histiocytes 'bleus'). *Annales d'Anatomie Pathologique*, **16**, 405.

COUDERC, P., DUFOUR, H., FAURE, H. & MARTIN, H. (1969) L'histiocytose lipidique de la rate au cours du purpura thrombopénique idiopathique. *Annales d'Anatomie Pathologique*, **14**, 309.

DOLLBERG, L., CASPER, J., DJALDETTI, M., KLOBANSKY, C. & VRIES, A. (1965) Lipid laden histiocytes in the spleen in thrombocytopenic purpura. *American Journal of Clinical Pathology*, **43**, 16.

DOSIK, H., ROSNER, F. & SAWITSKY, A. (1972) Acquired lipidosis: Gaucher-like cell and 'blue-cells' in chronic granulocytic leukemia. *Seminars in Hematology*, **9**, 309.

FERRANS, V.J., BUJA, M., ROBERTS, W. & FREDRICKSON, D.S. (1971) The spleen in type I hyperlipoproteinemia. Histochemical, biochemical, microfluorometric and electron microscopic observations. *American Journal of Pathology*, **64**, 67.

FORD, D.K., PRICE, G.E., CULLING, C.F.A. & VASSAR, P.S. (1962) Familial lipochrome pigmentation of histiocytes with hyperglobulinemia, pulmonary infiltration, splenomegaly, arthritis and susceptibility to infection. *American Journal of Medicine*, **33**, 478.

GEMSA, D., FUDENBERG, H.H. & SCHMID, R. (1974) Erythrophagocytosis: regulation of the heme-degrading enzyme system. In: *Activation of macrophages* (Ed. by W.H.Wagner, H.Hahn and R.Evans), p. 40. Excerpta Medica, Amsterdam.

GYÖRKEY, F., SHIMAMURA, T. & O'NEAL, R.M. (1967) The fine structure of ceroid in human atheroma. *Journal of Histochemistry and Cytochemistry*, **15**, 732.

HASHIMOTO, K. & PRITZKER, M.S. (1973) Electron microscopic study of Reticulohistiocytoma. *Archives of Dermatology*, **107**, 263.

HILL, J.M., SPEER, R.J. & GEDRIKOGLU, H. (1963) Secondary lipidosis of spleen associated with thrombocytopenia and other blood dyscrasias treated with steroids. *American Journal of Clinical Pathology*, **39**, 607.

HOWES, E.L., PRICE, H.M. & BLUMBERG, J.M. (1964) The effects of a diet producing lipochrome pigment (ceroid) on the ultrastructure of skeletal muscle in the rat. *American Journal of Pathology*, **45**, 599.

JACOBSEN, C.D., GJONE, E. & HOVIG, T. (1972) Sea-blue histiocyte in familial lecithin cholesterol acyltransferase deficiency. *Scandinavian Journal of Haematology*, **9**, 106.

JONES, B., GILBERT, E.F., ZUGIBE, F.T. & THOMPSON, H. (1970) Sea-blue histiocytes disease in siblings. *Lancet*, **ii**, 73.

KATTLOVE, H.E., WILLIANS, J.C., GAYNOR, E., SPIVACK, M., BRADLEY, R.M. & BRADY, P.O. (1969) Gaucher cells in chronic myelocytic leukemia: an acquired abnormality. *Blood. The Journal of Hematology*, **33**, 379.

KRISTENSON, K. & SOURANDER, P. (1966) Occurrence of lipofuxin in inherited metabolic disorders affecting the nervous system. *Journal of Neurology, Neurosurgery and Psychiatry*, **29**, 113.

LAKE, B.D., STEPHANS, R. & STEPHANS, N. (1970) Syndrome of the sea blue histiocyte (letter to the editor). *Lancet*, **ii**, 309.

LANDING, B.H., STRAUSS, L., CROCKER, A.C., BRAUSTEIN, H., HENLEY, W.L., WILL, J.R. & SANDERS, M. (1961) Thrombocytopenic purpura with histiocyte of the spleen. *New England Journal of Medicine*, **265**, 572.

LAUGIER, P., HUNZIKER, N., LAUT, J., ORUSCO, M. & OLMOS, L. (1975) Réticulo-histiocytose d'évolution bénigne (type Hashimoto-Pritzker). Etude en microscopie électronique. *Annales de Dermatologie et de Syphiligraphie*, **102**, 21.

LEE, R.E. & ELLIS, L.D. (1970) The storage cells of chronic myelogeneous leukemia. *Laboratory Investigation*, **24**, 261.

LOWDEN, J.A., BARSON, A.J. & WENTWORTH, P. (1970) Wolman's disease: a microscopic and biochemical study showing accumulation of ceroid and esterified cholesterol. *Canadian Medical Association Journal*, **102**, 402.

LUKES, R.J. & COLLINS, R.D. (1974) A functional approach to the classification of malignant lymphoma. In: *Diagnosis and therapy of malignant lymphoma* (Ed. by K.Musshoff), p. 18. Springer-Verlag, Berlin.

MARANOFF, R., FITE, F.K. & FRUMIN, A.M. (1972) Greenish blue granular marrow histiocyte associated with low serum cholesterol. *American Journal of Clinical Pathology*, **57**, 103.

OLMOS, L., & LAUGIER, P. (1974) La pigmentación cutànea en la amiodarona. Estudio ultrastructural. *Medicina Cutánea Ibero Latino Americana*, **2**, 447.

PIÑOL-AGUADÉ, J., PEYRI, J., FERRANDO, J., HERRERO, C., PEDRAGOSA, R., MIERAS, C., ESTRACH, T. & MASCARO, J.M. (1974) Reticulosis necróticas ? Una manifestación de la enfermedad de Scott y Robb-Smith ? *Medicina Cutánea Ibero Latino Americana*, **2**, 15.

QUATTRIN, N., DI GIROLAMO, R., MASTROBUONI, A. & MONTUORI, R. (1972) Una nuova malattia emorragica trombocitopenica: La 'istiocitosi blumare'. *Minerva Medica*, **63**, 1396.

QUINTON, S.W., WURZEL, H. & CZERNOBILSKY, B. (1967) Histiocytosis of spleen, lymph node, and bone marrow. Associated with thrombocytopenia, splenomegaly, and splenic angiomata. *American Journal of Clinical Pathology*, **47**, 484.

REYNÉS, M., KALIFAT, R. & DIEBOLD, J. (1973) Etude ultrastructurale des histiocytes bleu marine dans une lipidose splénique idiopathique avec surcharge en céroïdes. *Virchows Archiv; A Pathology Pathologische Anatomie*, **360**, 349.

REYNOLDS, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, **17**, 20.

RICHARDSON, K.C., JARETT, L. & FINKE, E.H. (1960) Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Technology*, **35**, 313.

RYWLIN A.M., HERNANDEZ, J.A., CHASTAIN, D.E. & PARDO, V. (1971a) Ceroid histiocytes of spleen and bone marrow in idiopathic thrombocytopenic purpura (ITP). A contribution to the understanding of the sea blue histiocyte. *Blood. The Journal of Hematology*, **37**, 587.

RYWLIN, A.M., LOPEZ-GOMEZ, A., TACHMES, P. & PARDO, V. (1971b) Ceroid histiocytosis of the spleen in hyperlipemia. *American Journal of Clinical Pathology*, **56**, 572.

SALMON, S.E. & SELIGMAN, M. (1974) B-cell neoplasia in man. *Lancet*, **ii**, 1230.

SALTZSTEIN, S.L. (1961) Phospholipid accumulation in histiocyte of splenic pulp associated with thrombocytopenic purpura. *Blood. The Journal of Hematology*, **18**, 73.

SAWITSKY, A., HYMAN, G.A. & HYMAN, J.B. (1954) An unidentified reticulo-endothelial cell in bone marrow and spleen. Report of two cases with histochemical studies. *Blood. The Journal of Hematology*, **9**, 977.

SCOTT, R.B. & ROBB-SMITH, A.H.T. (1939) Histiocytic medullary reticulosis. *Lancet*, **ii**, 194.

SILVERSTEIN, M.N., ELLEFSON, R.D. & AHERN, E.J. (1970) The syndrome of the sea blue histiocyte. *New England Journal of Medicine*, **282**, 1.

SIMON, G.T. & BURKE, J.S. (1970) Electron microscopy of the spleen: III, Erythroleukophagocytosis. *American Journal of Pathology*, **58**, 451.

SÖRENSEN quoted by CULLING, C.F.A. (1963) In: *Handbook of Histopathological Techniques* (Ed. by Butterworth and Co.), p. 160. London.

STEIN, H., LENNERT, K. & PARWARECH, M.R. (1972) Malignant lymphomas of B cell type. *Lancet*, **ii**, 855.

SUNDBERG, R.D., NELSON, D.A., HOILUND, L.J., HERBST, G.H. & BEECHER, N.B. (1964) Cell debris and blue pigment macrophages in chronic myelogenous leukemia. Abstract A. 37. X*th Congress International Society of Hematology, Stockholm.*

TERRY, R.D., SPERRY, W.H. & PRODOFF, B. (1954) A new lipidosis resembling Niemann-Pick's disease. *American Journal of Pathology*, **30**, 263.

WARNKE, R.A., KIM, H. & DORFMAN, R.F. (1975) Malignant histiocytosis (histiocytic medullary reticulosis). I. Clinicopathologic study of 29 cases. *Cancer*, **35**, 215.

WINKLER, H.H., FRAME, B., SAEED, S.M., SPINDLER, A.C. & BROUILLETTE J.N. (1969) Ceroid storage disease complicated by rupture of the spleen. *American Journal of Medicine*, **46**, 297.

ZAWADZKI, Z.A., PEÑA, C.E. & FISHER, E.R. (1969) Histiocytic medullary reticulosis. Case report with electron microscopic study. *Acta haematologica*, **42**, 50.

British Journal of Dermatology (1976) **95**, 45.

Leucosis and skin: acute lymphoid immunoblastic leukaemia

M.G.BERNENGO, A.ZINA AND G.ZINA

Istituto di Clinica Dermatologica, Università di Torino, Italy

Accepted for publication 21 November 1975

SUMMARY

The clinical course of a patient with acute lymphoid immunoblastic leukaemia and prominent nodular haemorrhagic skin lesions is described. Cytological, cytochemical and electronmicroscopic studies were performed on bone marrow and skin blast cells. The absence of surface immunoglobulins and of the other markers for B lymphocytes (EA and EAC rosettes) and the presence of 30% of spontaneous sheep erythrocyte rosettes excluding an acute leukaemia with Burkitt's tumour cells, suggest that T cells are involved. Complete haematological and cutaneous remission was obtained with prednisone therapy.

A recent classification of acute lymphoblastic leukaemias by Mathé *et al.* (1974) led to the observation of cells not covered by the descriptions given for acute lymphoblastic, myeloblastic and monoblastic forms. These were large blast cells with intensely basophilic (pyroninophilic) cytoplasm containing many vacuoles. Their morphological appearance was that of Burkitt lymphoma cells. Unlike these, however, they were not necessarily B cells. Mathé *et al.* described cases in which such cells were present as 'acute lymphoid immunoblastic leukaemia'. Other workers (Lennert, 1975; Lukes & Collins, 1974) have also shown that forms often classed as histioblastic or histiocytic lymphomas are in fact immunoblastic lymphomas.

Maculo-papular rashes are present in 40% of immunoblastic lymphoma cases (Frizzera, Moran & Rappaport, 1974; Lukes & Tindle, 1975). However they were not observed in the eight cases described by Mathé.

The present paper reports a case of acute immunoblastic leukaemia with specific skin lesions.

CASE REPORT

The patient was a 76-year-old male with no significant past history. In November 1974, he noted purpuric lesions on his forehead, face and trunk. These gradually increased in size and came together to form haemorrhagic suffusions. At the same time, roundish, nodular lesions 4·5 cm in diameter with well-defined borders appeared on his left temporal and sub-clavicular regions, and on his back. These nodules were covered with thin skin and were much infiltrated and haemorrhagic. They did not itch and were not painful (Fig. 1). On admission, the patient presented striking hepatosplenomegaly but no lymphadenopathy.

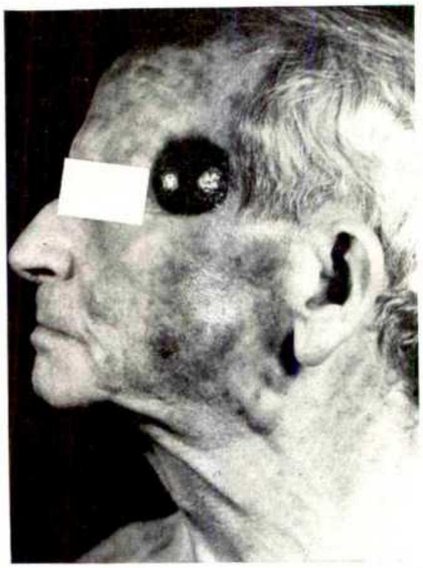


FIGURE 1. Roundish, nodular haemorrhagic lesion with diffuse purpura. Note the enlargement of neck lymph node.

The blood picture included WBC 8×10^3/mm^3, with a relative (40%) and absolute (3200/mm^3) lymphocytosis and 2% blasts. Haemoglobin 10 g/100 ml, RBC $3{\cdot}73 \times 10^6$/mm^3, platelets 168×10^3/mm^3. Normal prothrombin time, partial thromboplastin time, thrombin time, factors II, V, VII, VIII and IX, platelet ADP aggregation and thromboelastogram. The euglobulin lysis time was normal. Later, there was a slight fall in fibrinogen (170 mg%) titre and in factor V (70%). There was slight increase in fibrin degradation products (FDP), the protamine sulphate paracoagulation test was slightly positive for FDP. The thromboelastogram now included lengthening of R and K. Platelets decreased to 90×10^3/mm^3. The overall picture was one of consumptive coagulopathy.

X-ray of the chest showed no signs of mediastinal adenopathy or parenchymal alterations. X-ray of the digestive tract was normal, except for signs of gastroduodenitis. Total serum proteins were 5·8 g/100 ml with 3 g/100 ml albumin and 2·8 g/100 ml globulin. Immunoelectrophoresis: absence of paraproteins. Immunodiffusion: IgG 624 mg%, IgA 95 mg% ml, IgM 77 mg% ml. Repeated urinary examination did not reveal proteinuria or Bence–Jones proteins. Sternal puncture showed a hypercellular marrow, with erythroblasts well in evidence; 80% of the cells were large blasts with intensely basophilic cytoplasm. Some cells had numerous peripheral vacuoles.

The histological picture showed a thin epidermis with a linear profile and frequent Malpighian vacuolar degeneration and abundant dermal infiltration by blast cells. There was abundant haemorrhage and blood pigment deposits. The morphological resemblance of the skin cell imprints to marrow cells was corroborated by their complete cytochemical correspondence (Figs 2 & 3).

After a few days of therapy with prednisone there was complete regression of the nodules and reduction of purpura; almost complete resolution of the generalized lymphadenopathy was noted a month after admission.

After 8 months the patient is still well. Neither lymphadenopathy nor skin lesions are detected. Haemoglobin 13·4g%, RBC $4{\cdot}59 \times 10^6$/mm^3, WBC 6×10^3/mm^3, (neutrophils 70%, lymphocytes 26%, monocytes 4%), platelets $1{\cdot}6 \times 10^5$/mm^3, fibrinogen 225 mg%, euglobulin lysis time and fibrinogen degradation products normal, protamine sulphate paracoagulation test negative. Total serum proteins

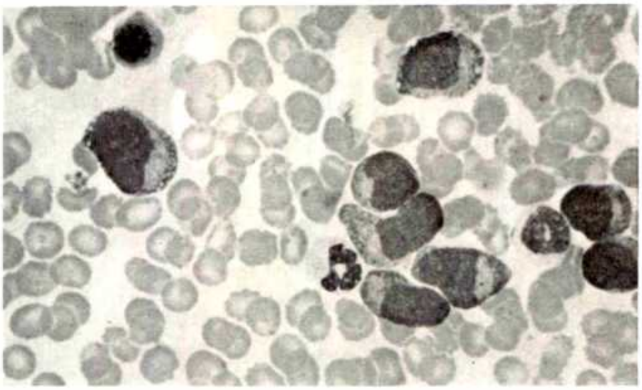


FIGURE 2. Leukaemic immunoblasts in bone marrow. Note the numerous peripheral vacuoles (May–Grünwald–Giemsa, × 1000).

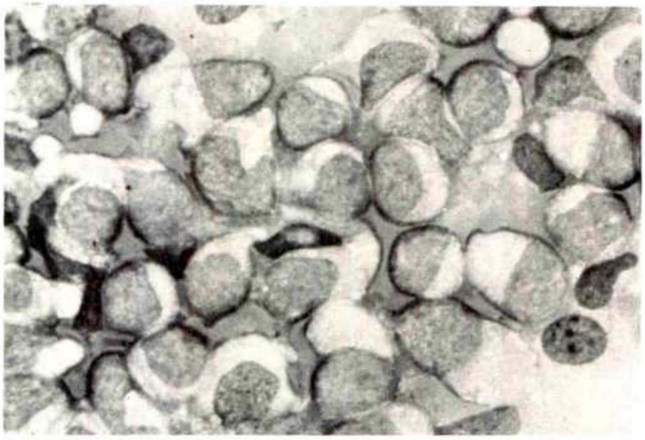


FIGURE 3. Skin cell imprints (May–Grünwald–Giemsa, × 1000).

are 6·8 g/100 ml with 14% globulin. Immunodiffusion: IgG 785 mg%, IgG 140 mg%, IgM 127 mg%.

MATERIAL AND METHODS

Cytological and cytochemical studies. Films of peripheral blood and films of needle aspirates of bone marrow obtained from the sternum were made and stained with May–Grünwald–Giemsa. Cytochemical stains, carried out on skin biopsies as well as on films of peripheral blood and bone marrow from the patient, included peroxidase reaction (with Kaplow's techniques, Hayhoe, Quaglino & Doll, 1964), Sudan Black B reaction (with the method of Sheehan & Storey, 1947), periodic acid-Schiff reaction (according to Hayhoe *et al.*, 1964), α-naphthyl-esterase (Rozenszayn *et al.*, 1968), double esterase (naphthol-AS-acetate plus naphthol-AS-D-chloroacetate according to Yam, Li & Crosby, 1971) and acid phosphatase (according to Barka & Anderson, 1962).

Immunological studies: rosette assay, E, EA, and EAC rosettes for quantification of T and B cells, respectively, were determined according to the methods described by Wybran, Carr & Fudenberg (1972) and Bianco, Patrick & Nussenzweig (1970).

Staining with fluorescein-labelled anti-immunoglobulin. Peripheral blood lymphocytes separated by the Ficoll-Hypaque method, and blast cells obtained from bone marrow aspirates and from skin biopsies, were incubated with monovalent fluorescein-conjugated rabbit-anti-human immunoglobulin serum, (anti-IgG, IgM, IgA, IgD and IgE) for 45 min at 4°C according to Pernis, Forni & Amante (1970). The cellular suspension was washed three times with Hanks' solution at 4°C. Fluorescence was read with a Leitz microscope equipped with a mercury vapour lamp and fluorescence vertical illuminator.

Electron microscopy. The biopsies were fixed in 3% glutaraldehyde in phosphate buffer, post-fixed in 1% buffered osmium tetroxide to which sucrose had been added. The tissues were dehydrated in graded alcohols and embedded in Araldite. Sections were cut on a Reichert ultramicrotome and then double stained with uranyl acetate and lead citrate (according to Reynolds, 1963). The sections were viewed by means of a 'Siemens Elmiscope II'.

RESULTS

Cytochemistry. Peroxidase, Sudan black B reaction, non-specific esterases (α-naphtyl-acetate and naphthol-AS-acetate esterases), naphthol-AS-D-chloracetate esterase and acid phosphatases were negative in blast cells. In all the cells PAS positivity was intense with positive granules and blocks against a negative cytoplasmic background. In addition the blasts were intensely pyroninophilic.

Immunological examination. Bone marrow, peripheral and skin blast cells revealed absence of surface membrane immunoglobulins in immunofluorescence assay. No receptors for the third component of complement (C_3) were detected in blast cells by the EAC rosette test. Thirty percent of marrow blasts had the capacity to bind sheep red blood cells to their surfaces (rosette E). The percentage of rosette E in peripheral lymphocytes was 69%, EA 6·5%, EAC 9%, IgG 1%, IgA 2%, IgM 2·5%, IgD 3%, IgE 1·5%.

Electron microscopy. Electron microscopy of bone marrow and of skin cells confirmed the cytochemical results indicating that the cells belonged to the lymphocytic series and excluded an acute monocytoid or myelomonocytic leukaemia. The cells showed a nucleus with prominent nucleolus, abundant ribosome- and polyribosome-rich cytoplasm, mitochondria and a well-developed Golgi apparatus.

DISCUSSION

In this case the underlying blood disorder was revealed by the onset of the dermatosis. While frequently purpura and skin lesions may be the presenting feature of a leukaemia, the present case is unusual in that cells of the dermal infiltrate belong to a new type of leukaemia in which the cells differ from those of acute myeloid and monoblastic leukaemia but also differ from the lymphoid types described by Mathé: microlymphoblastic, macrolymphoblastic, prolymphocytic and prolymphoblastic. Myeloblastic leukaemia could be excluded because peroxidase, Sudan black and naphthol-AS-D-chloroacetate esterase staining gave negative results. Negativity of Naphthol-A S-D acetate and of α-naphthyl-acetate esterases, and intense PAS positivity with coarse granules and blocks eliminated the possibility of an acute monoblastic leukaemia.

Electron microscopy confirms the lymphocytic origin of the blast cells studied and may suggest an immunoblastic type. Hypogammaglobulinaemia, absence of receptors for immunoglobulins or C_3 on the cell membrane and the 30% of spontaneous rosettes with sheep red blood cells exclude Burkitt's lymphoma. In addition, the blasts differed from the prolymphoblastic type insofar as they had many vacuoles and intensely pyroninophilic cytoplasm.

Our immunological data suggest that T cells may be involved. Mathé *et al.* found that the immunoblastic cells may carry the T or B or no membrane immunological markers.

One form of acute lymphocytic leukaemia observed by Lukes & Collins (1974) may involve T cells and is composed of a distinctive convoluted lymphocytic proliferation. Lukes' lymphocytic leukaemia only affects adolescents and the age of our patient (76 years) would therefore exclude such a diagnosis. The morphological appearance of the cells also contradicts this view.

In conclusion by cytochemical, morphological, immunological and electron microscopic results we can describe this case as 'acute lymphoid immunoblastic leukaemia with specific skin lesions'. Diagnosis of skin lesions of this type must thus take into account acute lymphoid immunoblastic leukaemia, in addition to other known leukaemia types. This distinction is based on morphological, cytochemical and electronmicroscopic evidence. Moreover, its recognition is of practical importance, since the minimum possible resort to cytotoxic drugs is essential when treating this new morbid form: in fact deaths are usually caused by overwhelming infections. In immunoblastic lymphadenopathy also steroids are considered the drugs of choice (Frizzera *et al.*, 1975), since cytotoxic drugs may merely increase susceptibility to intercurrent infection, and so accelerate the course of the disease.

REFERENCES

Barka, T. & Anderson, P.J. (1962) Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *Journal of Histochemistry and Cytochemistry*, 10, 741.

Bianco, C., Patrick, R. & Nussenzweig, V. (1970) A population of lymphocytes bearing a membrane receptor for antigen–antibody–complement complexes. I. Separation and characterization. *Journal of Experimental Medicine*, 132, 702.

Frizzera, G., Moran, E.M. & Rappaport, H. (1974) Angio-immunoblastic lymphadenopathy with dysproteinaemia. *Lancet*, i, 1070.

Hayhoe, F.G.J., Quaglino, D. & Doll, R. (1964) The cytology and cytochemistry of acute leukaemias. *MRC Special Report Series no*, 304, p. 16. H.M.S.O., London.

Lennert, K. (1975) New criteria for the classification of malignant lymphomas. *British Journal of Cancer*, 31, Suppl. II, 29.

Lukes, R.J. & Collins, R.D. (1974) Immunologic characterization of human malignant lymphomas. *Cancer*, 34, 1488.

Lukes, R.J. & Tindle, B.H. (1975) Immunoblastic lymphadenopathy; a hyperimmune entity resembling Hodgkin's Disease. *New England Journal of Medicine*, 292, 1.

Mathé, G., Belpomme, D., Dantchev, D., Pouillart, P., Jasmin, C.L., Misset, J.L., Musset, M., Amiel, J.L., Schlumberger, J.R., Schwarzenberg, L., Hayat, M., De Vassal, F. & Lafleur, M. (1974) Immunoblastic acute lymphoid leukaemia. *Biomedicine*, 20, 333.

Pernis, B., Forni, L. & Amante, L. (1970) Immunoglobulin spots on the surface of rabbit lymphocytes. *Journal of Experimental Medicine*, 132, 1001.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cellular Biology*, 17, 208:

Rozenszajn, L., Leibovich, M., Shoam, D. & Epstein, J. (1968) The esterase activity in megaloblasts, leukaemic and normal haemopoietic cells. *British Journal of Haematology*, 14, 605.

Sheehan, H.L. & Storey, G.W. (1947) An improved method of staining leukocyte granules with Sudan black. *British Journal of Pathology and Bacteriology*, 59, 336.

Wybran, J., Carr, M.C. & Fudenberg, H.H. (1972) The human rosette forming cell as marker of population of thymus-derived cells. *Journal of Clinical Investigation*, 51, 2537.

Yam, L.T., Li, C.Y. & Crosby, W.H. (1971) Cytochemical identification of monocytes and granulocytes. *American Journal of Clinical Pathology*, 55, 283.

British Journal of Dermatology (1976) 95, 51.

Hypersensitivity of scleroderma cutaneous vascular smooth muscle to 5-hydroxytryptamine

R.K. WINKELMANN, MARC E. GOLDYNE AND RONALD L. LINSCHEID
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901, U.S.A.

Accepted for publication 30 October 1975

SUMMARY

Vascular smooth muscle strips from subcutaneous wrist vessels of patients with scleroderma (systemic sclerosis) were studied in an isolated system. No difference between catecholamine response of scleroderma or control vessel strips was observed. Three of four patients demonstrated significant hypersensitivity to 5-hydroxytryptamine as compared to control values. Serotonin hypersensitivity may play a role in the evolution of Raynaud's phenomenon and vascular scleroderma.

Studies of blood flow in the skin of the extremities of patients with scleroderma (systemic sclerosis) have demonstrated decreased circulation, which is confirmed by decreased heat loss and oxygenation of the sclerodermatous skin (LeRoy, Downey & Cannon, 1971; Winkelmann, 1971). Because 95% of patients with scleroderma have Raynaud's phenomenon (prolonged cold vasoconstriction), it has been assumed that sympathetic nerve hyperactivity, an excess of catecholamine or of other mediator, a structural vascular lesion, or all three could account for the decreased circulation and the sensitivity to cold (Lewis, 1936). Sympathectomy alone does not reverse Raynaud's phenomenon and, by itself, is associated with hypersensitivity to catecholamine (Duff, 1952). Vascular structural lesions have been observed in the digital vessels of patients with scleroderma (Lewis, 1929). A recent study confirmed the fibromucinous endothelial proliferation in the subcutaneous vessels of the wrist of patients with scleroderma and demonstrated alterations in catecholamine response to cold (Winkelmann, Goldyne & Linscheid, submitted for publication). One other mediator that causes vasoconstriction is 5-hydroxytryptamine (serotonin), and Halpern *et al.* (1960) demonstrated that the intra-arterial injection of 5-hydroxytryptamine produced prolonged digital vasoconstriction, similar to that of Raynaud's phenomenon. Our current study with isolated vascular smooth-muscle strips from the skin of patients with scleroderma demonstrated a hypersensitivity to 5-hydroxytryptamine that is apparently not related to the structural vascular lesion and may be a contributing factor in Raynaud's phenomenon.

MATERIALS AND METHODS

A collateral branch of the radial artery coursing to the thenar eminence was excised in each of four

Reprint requests to Dr Winkelmann, Mayo Clinic, Rochester, Minnesota 55901, U.S.A.

patients with scleroderma. The outer diameters of the vessels ranged from 800 to 1000 μm. All four small vessels were easily dissected from the connective tissue and were normal on gross inspection. A small cross-section was preserved for histological study, and the remainder of the vessel was cut into helical strips for *in vitro* pharmacological testing. When the pharmacological studies were completed, the strips were examined histologically. Six vessel strips from the four patients were studied. Control vessels were excised from three patients at the time of wrist surgery for nonsclerodermatous conditions. Six strips were prepared and studied histologically and by *in vitro* pharmacological testing.

The patients with scleroderma all had the acrosclerosis form of systemic scleroderma. Raynaud's phenomenon and cutaneous sclerosis were present in all four female patients whose ages ranged from 43 to 60 years and whose duration of disease was from 5 to 10 years. Oesophageal changes of scleroderma were present in all, and pulmonary function and carbon monoxide diffusing capacity were diminished in three of the four patients. Two patients had intestinal and colon diverticula. The erythrocyte sedimentation rate was, respectively, 10, 13, 35 and 45 mm in 1 h (Westergren) at the time of the study. One patient had an associated Sjögren's syndrome with an elevated level of γ-globulin and a positive rheumatoid factor. Three patients, including the one with Sjögren's syndrome, had positive serum antinuclear antibodies.

METHODS

The vessels were dissected free from the subcutaneous tissue, and helical strips were cut and affixed in a bath of physiological salt solution charged with 95% oxygen and 5% carbon dioxide (Winkelmann, Sams & Bohr, 1969). The composition (meq/l) of the physiological salt solution was: NaCl, 119·0; KCl, 4·7; KH_2PO_4, 1·18; $MgSO_4$, 1·17; $NaHCO_3$, 14·9; glucose, 5·5; sucrose, 50·0; $CaCl_2$, 1·6; and calcium disodium EDTA (Versenate) 0·026 (to chelate trace metals that would catalyse autooxidation of catecholamines). One end of each strip was attached with 6–0 silk suture to a fixed point, and the other was attached to the movable arm of a force displacement transducer (Grass Model FTO3C). Contractions were recorded with a polygraph (Grass Model 5) at 0·25 mm/s. The muscle strip was placed under 100 mg of tension and permitted to equilibrate for 2 h, during which time it was washed with physiological salt solution every 15 min. Noradrenaline bitartrate monohydrate (Levophed), adrenaline hydrochloride, isoproterenol hydrochloride (Isuprel), 5-hydroxytryptamine creatinine sulphate (Sigma), bradykinin triacetate trihydrate (Calbiochem), and prostaglandin E_2 were used*.

The concentration of drugs used is reported as the final concentration in the bath in g/ml. The duration of exposure to test substances was 1 min if the response was immediate or as long as 5 min if the response was slow.

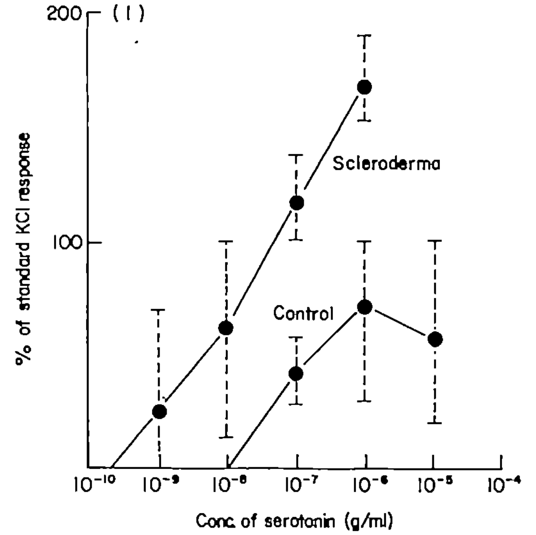
The vessel-muscle strips were studied for response to potassium chloride, and a dose–response curve to potassium chloride was constructed. Similar dose–response curves to adrenaline or noradrenaline (or both) and to 5-hydroxytryptamine were studied. Threshold response and dose–response curves to isoproterenol, prostaglandin E_2, ATP, and bradykinin also were studied.

RESULTS

The response of three of the four patients to 5-hydroxytryptamine was increased over control values and the threshold of response was lowered (Fig. 1). The dose–response curves of the three hyperresponding patients are compared to the dose–response curves of the control normal tissue. Two

---

* Crystalline prostaglandin E_2 was kindly supplied by Dr John Updike, Upjohn Company.



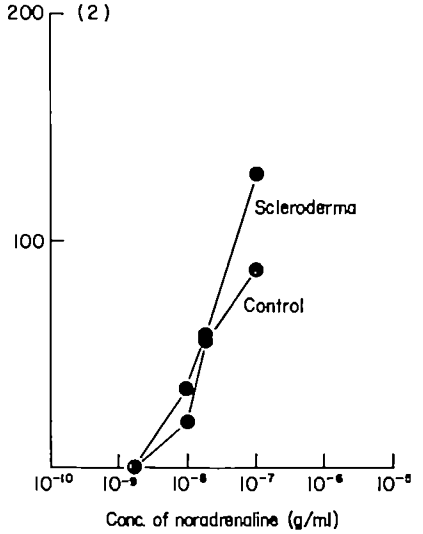


FIGURE 1. Dose response to 5-hydroxytryptamine by wrist vessel strips in three patients with scleroderma and three controls.

FIGURE 2. Dose response to noradrenaline by control and scleroderma wrist vessel strips.

vessel strips from the fourth patient gave a response that coincided with the values of the control group.

Catecholamine sensitivity of the vessel strips from patients with scleroderma and controls was essentially identical. There was no change in the threshold or the degree of responsiveness. The dose-response curves were essentially similar (Fig. 2).

The responses to isoproterenol and ATP were similar in both sclerodermatous and control tissue. Low contraction responses occurred to high concentrations only (10^{-5}–10^{-4} g/ml). Prostaglandin E_2 contraction response occurred at 10^{-7}–10^{-6} g/ml in both sclerodermatous and control tissue, producing similar low-dose response curves in all experiments. In two experiments, bradykinin (10^{-4} g/ml) gave no response with sclerodermatous vascular smooth muscle.

The blood vessels from all four patients demonstrated endothelial fibrous proliferation. The internal elastic lamina was normal and intact. The media and adventitial layers were normal. No inflammation was observed. Mucinous degeneration was observed in the fibrous endothelial mass. Examination of the longitudinal strips after pharmacological study revealed the same fibrous endothelial change. Control vessels did not show this histological alteration.

COMMENT

This study supports a role for 5-hydroxytryptamine in the vascular changes of scleroderma. The increased reactivity to this mediator found in the platelets could be an associated cause of the prolonged vessel contraction with various stimuli. Halpern *et al.* (1960) found that the intra-arterial injection of 5-hydroxytryptamine produced the same skin temperature changes as observed in Raynaud's phenomenon and noted that delays in 5-hydroxytryptamine destruction in the blood or receptor blockade affected Raynaud's phenomenon in a predictable manner. Scherbel & Harrison (1959) noted that the subcutaneous injection of 5-hydroxytryptamine produced prolonged vasoconstriction in sclerodermatous hands and it was believed to be the small subcutaneous vessels that were hyperreactive. 5-Hydroxytryptamine produced fibrosis when injected subcutaneously (MacDonald, Robbins & Mallory, 1958), and a form of unusual cutaneous 'scleroderma' has been described in the carcinoid syndrome (Fries, Lindgren & Bull, 1973). Drugs such as methysergide (a 5-hydroxytryptamine antagonist) and practolol (a β-blocking agent) have been associated with fibrosis, indicating that vasoactive mediators and their blocking agents can affect vessels and subcutaneous and dermal tissue. Boucek & Nobel (1973) have reported a 5-hydroxytryptamine-stimulating effect on mouse and human fibroblast cultures and noted that noradrenaline potentiates this effect of 5-hydroxytryptamine.

Roddie, Shepherd & Whelan (1955) reported that the intra-arterial infusions of 5-hydroxytryptamine cause greater vasoconstriction in the hand than in the forearm. Fox *et al.* (1961) and Daugherty *et al.* (1968) also reported a vasoconstrictor effect on cutaneous vessels. Abboud (1968) pointed out that, in the canine paw, 5-hydroxytryptamine had a selective vasoconstrictor effect on veins and arteries larger than on the metacarpal artery. It has also been pointed out that the intra-arterial injection of 5-hydroxytryptamine does not cause increased microvascular permeability and oedema in the canine paw (Merrill *et al.*, 1974).

Gaddum (1953) described a specific receptor for serotonin, or 5-hydroxytryptamine, that was separate from catecholamine, histamine, or acetylcholine receptors. We believe that the 5-hydroxytryptamine receptor is a significant and common receptor for cutaneous vessels because it was found in all areas of dog, rabbit and human skin studied (Sundt & Winkelmann, 1972; Winkelmann, Sams & King, 1973). Other receptors vary with species and region of skin studied and most particularly in different organs (Bohr, Goulet & Taquini, 1961).

The hyperactivity of the 5-hydroxytryptamine receptor may be caused by the physical or chemical change at the membrane or by an alteration of another receptor. A synergistic activity between mediators may exist. Because catecholamine sensitivity of sclerodermatous cutaneous vessels is rapidly lost as the temperature is lowered (Winkelmann, Goldyne & Linscheid, submitted for publication), this may have some capacity to augment the receptor response to 5-hydroxytryptamine. Lowering of catecholamine response produced by cold might induce the elevated receptor response to 5-hydroxytryptamine. The sclerodermatous tissue is poorly oxygenated because of poor circulation, and it has been shown that 5-hydroxytryptamine response in human umbilical arteries has a direct linear relation to PO_2 (Nair & Dyer, 1973).

The capacity of other mediators or factors to augment catecholamine or 5-hydroxytryptamine responses must be considered. Potentiation of cutaneous smooth-muscle activity to catecholamine is is produced *in vitro* by prostaglandin E_2 (Goldyne & Winkelmann, 1973). Other authors have demonstrated that PGF_2 (Greenberg *et al.*, 1973) and PGE (Khairallah, Page & Türker, 1967) potentiated the response to 5-hydroxytryptamine. Studies of similar potentiation must be performed with cutaneous vessel strips in scleroderma. Changes in 5-hydroxytryptamine response with temperature must be studied: one preliminary experiment confirmed 5-hydroxytryptamine hyperactivity at 37°C but failed to show a further increase in hyperactivity of the dose–response curve at 27°C.

The presence of this 5-hydroxytryptamine hyperreactivity in three of our patients and its absence in the two vessel strips from our fourth patient does not correlate with vessel disease that showed endothelial fibrosis in all vessels of all four patients. We conclude that the 5-hydroxytryptamine sensitivity is independent of, and perhaps not the result of, this pathological change.

The vessel strips of the fourth patient responded normally to 5-hydroxytryptamine, and the clinical, pathological or immunological status of this patient did not differ from that of the other patients. The fourth patient had taken no drugs of significance but drank 1 quart of whisky each week. The scleroderma disease in this patient was similar in degree of involvement and duration (7 years) to that of the other patients (5–10 years). All four patients are alive with progressive disease.

Hyperresponsiveness to 5-hydroxytryptamine has been proposed as a cause of migraine (Sicuteri, Anselmi & Del Bianco, 1973). We believe that the 5-hydroxytryptamine hyperresponsiveness, as well as the sympathetic hyperresponsiveness of some patients, may be a significant contributing factor to the vascular as well as to the fibrous changes of the disease, and both must be considered in the control of Raynaud's phenomenon and scleroderma. For an individual patient, control of either of the hyperreactivities may be of clinical significance; in some patients, both may be important. Because of the structural lesion of the vessel, treatment of either hyperreactivity probably will not reverse the process, but control of the progression of the symptoms of Raynaud's phenomenon can be anticipated. The observed receptor hyperactivity supports the concept that further study of the tissue may indicate appropriate ways to block or modify receptor behaviour and through this mechanism to control the disease.

REFERENCES

Abboud, F.M. (1968) Vascular responses to norepinephrine, angiotensin, vasopressin and serotonin. *Federation Proceedings; Federation of American Societies for Experimental Biology*, **27**, 1391.

Bohr, D.F., Goulet, P.L. & Taquini, A.C., Jr (1961) Direct tension recording from smooth muscle of resistance vessels from various organs. *Angiology*, **12**, 478.

Boucek, R.J. & Noble, N.L. (1973) Histamine, norepinephrine and bradykinin stimulation of fibroblast growth and modification of serotonin response. *Proceedings of the Society for Experimental Biology and Medicine*, **144**, 929.

Daugherty, R.M., Jr, Scott, J.B., Emerson, T.E., Jr & Haddy, F.J. (1968) Comparison of iv and ia infusion of vasoactive agents on dog forelimb blood flow. *American Journal of Physiology*, **214**, 611.

Duff, R.S. (1952) Effect of sympathectomy on the response to adrenaline of the blood vessels of the skin in man. *Journal of Physiology (London)*, **117**, 415.

Fox, R.H., Goldsmith, R., Kidd, D.J. & Lewis, G.P. (1961) Bradykinin as a vasodilator in man. *Journal of Physiology (London)*, **157**, 589

Fries, J.F., Lindgren, J.A. & Bull, J.M. (1973) Scleroderma-like lesions and the carcinoid syndrome. *Archives of Internal Medicine*, **131**, 550.

Gaddum, J.H. (1953) Tryptamine receptors. *Journal of Physiology (London)*, **119**, 363.

Goldyne, M.E. & Winkelmann, R.K. (1973) *In vitro* effects of prostaglandin E_2 on cutaneous vascular smooth muscle in the dog and in man. *Journal of Investigative Dermatology*, **60**, 258.

Greenberg, S., Kadowitz, P.J., Diecke, F.P.J. & Long, J.P. (1973) Effect of prostaglandin $F_{2}\alpha$ on responses of vascular smooth muscle to serotonin, angiotensin and epinephrine. *Archives Internationales de Pharmacodynamie et de Therapie*, **206**, 5.

Halpern, A., Kuhn, P.H., Shaftel, H.E., Samuels, S.S., Shaftel, N., Selman, D. & Birch, H.G. (1960) Raynaud's disease, Raynaud's phenomenon and serotonin. *Angiology*, **11**, 151.

Khairallah, P.A., Page, I.H. & Türker, R.K. (1967) Some properties of prostaglandin E_1 action on muscle. *Archives Internationales de Pharmacodynamie et de Therapie*, **169**, 328.

LeRoy, E.C., Downey, J.A. & Cannon, P.J. (1971) Skin capillary blood flow in scleroderma. *Journal of Clinical Investigation*, **50**, 930.

Lewis, T. (1929) Experiments relating to the peripheral mechanism involved in spasmodic arrest of the circulation in the fingers: a variety of Raynaud's disease. *Heart*, **15**, 7.

Lewis, T. (1936) *Vascular Disorders of the Limbs: Described for Practitioners and Students*. Macmillan, New York.

MacDonald, R.A., Robbins, S.L. & Mallory, G.K. (1958) Dermal fibrosis following subcutaneous injections of serotonin creatinine sulphate. *Proceedings of the Society for Experimental Biology and Medicine*, **97**, 334.

Merrill, G.F., Kline, R.L., Haddy, F.J. & Grega, G.J. (1974) Effects of locally infused serotonin on canine forelimb weight and segmental vascular resistances. *Journal of Pharmacology and Experimental Therapeutics*, **189**, 140.

Nair, X. & Dyer, D.C. (1973) Effect on metabolic inhibitors and oxygen on responses of human umbilical arteries. *American Journal of Physiology*, **225**, 1118.

Roddie, I.C., Shepherd, J.T. & Whelan, R.F. (1955) The action of 5-hydroxytryptamine on the blood vessels of the human hand and forearm. *British Journal of Pharmacology*, **10**, 445.

Scherbel, A.L. & Harrison, J.W. (1959) Response to serotonin and its antagonists in patients with rheumatoid arthritis and related diseases. *Angiology*, **10**, 29.

British Journal of Dermatology (1976) 95, 57.

The prognosis of systemic sclerosis

N.R.ROWELL

Department of Dermatology, General Infirmary at Leeds

Accepted for publication 24 October 1975

SUMMARY

Eighty-four patients with systemic sclerosis have been investigated and observed at intervals for up to 15 years. The prognosis is worse in males than in females although in any individual case the clinical course is unpredictable. Those patients with the combination of calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia have the same degree of systemic involvement and the same prognosis as other patients with systemic sclerosis, and the term 'CRST syndrome' should be abandoned.

Recent interest in the evaluation of treatment of systemic sclerosis has underlined the importance of considering the natural history of the disease.

Classification

There has been considerable confusion in the nomenclature of scleroderma in the past. The following classification is now widely accepted. 1. Morphoea, which can be (a) localized in the form of plaques or linear lesions, or (b) generalized and involve the face, trunk and limbs. Raynaud's phenomenon and systemic changes are usually absent, and the lesions tend to improve in the course of years. 2. Systemic sclerosis, in which Raynaud's phenomenon nearly always occurs and is associated with sclerotic changes in the face, hands and, to a lesser extent, the feet. The heart, lungs, gastro-intestinal tract, kidneys and other organs can be involved.

The term 'diffuse systemic scleroderma' has been given by Tuffanelli & Winkelmann (1962) to those cases without Raynaud's phenomenon, involving the trunk rather than the face and hands, and with a poor prognosis. The sex ratio is said to be equal. Tuffanelli & Winkelmann (1961) found 39 of 727 patients (5·4%) in this category. This type of case is very rare in the United Kingdom.

Morphoea and systemic sclerosis can occur in the same patient. Changes of the former improve over the years while those of the latter persist or increase. This occurred in two patients of the present series.

Eighty-four patients (63 female, 21 male) with systemic sclerosis have been observed over the past 15 years. Their ages ranged from 21 to 74 years and all but three had Raynaud's phenomenon. The incidence of systemic involvement is shown in Table 1.

RESULTS

In those patients where the interval between the onset of Raynaud's phenomenon and the onset of cutaneous changes could be accurately assessed, the mean time in females (5·1 ± 7·0 years) was much longer than in males (0·25 ± 0·8 years). This is highly significant ($P < 0{\cdot}005$). This sex difference in

TABLE 1. Incidence of major systemic involvement in eighty-four patients with systemic sclerosis

		%
Oesophagus	(radiological)	73
Heart	(abnormal E.C.G.)	49
Lungs	(radiological)	46
Kidney	(abnormal creatinine clearance)	41

TABLE 2. Interval between the onset of Raynaud's phenomenon and the onset of cutaneous changes of systemic sclerosis when these could be dated

Interval (years)	Male	Female	Total
0–2	17	23	40
3–4	0	6	6
5–6	0	6	6
7–8	0	2	2
9–30	0	8	8
	17	45	62

Dichotomized as shown $\chi^2 = 10{\cdot}84$: d.f. = 1; $P < 0{\cdot}001$.

the speed of progress is even more strikingly demonstrated in Table 2 which shows that all the males had developed cutaneous signs within 2 years of the onset of Raynaud's phenomenon, whereas the interval in females was as long as 29 years.

Seven out of twenty-one males (33%) died in the period of study compared with fifteen of sixty-three females (24%). The duration between the onset of Raynaud's phenomenon and death in fatal cases is longer in females (11·0 ± 8·9 years) than in males (9·1 ± 4·4 years). Further cases are required to confirm that these differences are significant. The causes of death are shown in Table 3.

There was no alteration in prognosis in those patients who developed Raynaud's phenomenon or cutaneous changes after the age of 45 years as compared with those who developed these features before this age. Nor was there any relation of the natural history to the erythrocyte sedimentation rate, the presence or absence of antinuclear factor or to the type of antinuclear factor. The titres of antinuclear factor were estimated every 3–6 months and did not vary markedly with the clinical progress. The results of tests for antinuclear factor in fatal cases are shown in Table 4.

Twelve patients, all female, had the combination of calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia described as a syndrome with a good prognosis by Winterbauer (1964). All had involvement of the internal organs characteristic of systemic sclerosis and two died of the disease. Table 5 shows that the mean interval between the onset of Raynaud's phenomenon and the onset of cutaneous changes, where these could be dated, does not differ between patients with this so-called CRST group and patients without these features.

The unpredictability of the clinical course is illustrated by the following examples.

A 41-year-old married woman developed Raynaud's phenomenon and increasing stiffness and

TABLE 3. Cause of death in twenty-three fatal cases of systemic sclerosis

Respiratory failure and bronchopneumonia	9
Gastrointestinal including perforations	4
Malignant hypertension and renal failure	4
Other cause:	
Aplastic anaemia and S.L.E.	1
Anaemia and septicaemia	1
Pulmonary embolism	1
Unknown	3

TABLE 4. Antinuclear antibody in fatal cases

	Present	Absent
Male	4	2
Female	9	3
Total	13*	5

*H 6; S 0; N 1; H+S 3; H+N 2; H+S+N 1. (H = homogeneous; S = speckled; N = nucleolar.)

TABLE 5. Mean interval between onset of Raynaud's phenomenon and the onset of cutaneous changes in the 'CRST syndrome' ? in the whole series of females and in females without this combination of features

	'CRST'	Whole series of females	Females without 'CRST'
n	10	45	35
Mean	6·8	5·0	4·4
s.d.	8·7	7·0	6·5

CRST vs Whole series, $P = <0{\cdot}25$; CRST vs Females without CRST, $P = <0{\cdot}20$; Whole series vs Females without CRST, $P = <0{\cdot}35$.

sclerosis of the hands, feet, face and upper chest early in 1963. Complete investigation of every system in September 1963 showed no abnormality apart from some widening of the periodontal membrane. In particular, blood pressure and renal function tests were normal. Seven months later, in April 1964, she was admitted with malignant hypertension and died within 4 weeks. At autopsy only the skin and kidneys showed pathological changes of systemic sclerosis.

An explosive onset with gangrene does not necessarily imply a rapid death. A woman with acrosclerosis lost several fingers from gangrene at the age of 18 years. Extensive investigations at the age of 52 years showed that the only systemic change was some dilatation of the third part of the duodenum.

The male sex does not always indicate a poor prognosis. A man, now aged 55 years, developed acrosclerotic changes in the face and hands at the age of 37 and investigation at 40 showed him to have changes in the lower oesophagus, diverticula of the colon, impaired renal function and gross fibrotic changes in the lungs with pulmonary diffusion 32% of predicted normal. He is still working 15 years later.

DISCUSSION

The present investigation has shown that the clinical progress in systemic sclerosis is speeded up in males as compared with females and the prognosis tends to be poorer in males. The sex difference in prognosis has also been noted in the United States by Medsger *et al.* (1971) but is not widely known. These workers, using a life-table-analysis technique, showed that women survived significantly better than men. Males also tended to have more organs involved. The poorer prognosis of males compared with females is also found in systemic lupus erythematosus (Kellum & Haserick, 1964). It has been argued that these differences in progress and prognosis in both diseases occur because males have a poorer endogenous defence mechanism controlling the disease process because of having only one X chromosome (Burch & Rowell, 1963, 1965).

Medsger *et al.* (1971) also found that older patients (i.e. over 45 years on entry to his series) fared significantly worse than younger patients. Gastrointestinal tract involvement did not significantly alter survival but survival was limited by pulmonary, cardiac and, particularly, renal involvement. The poorer prognosis of cases with extensive skin changes was due to associated visceral involvement. The present series does not show that patients with late onset of Raynaud's phenomenon or of cutaneous changes have a poorer prognosis.

The overall cumulative survival in Medsger *et al.*'s (1971) series was 48% at 5 years and 35% at 7 years. Bennett *et al.* (1971) in this country also used a life-table method and obtained similar results in respect of the effect of age and systemic involvement. However, they did not observe the effect of sex on prognosis. Their 5-year survival rate overall was 73% which is better than other series. This is probably due to the fact that they had a higher proportion of females in their series. Of those dying, 73% did so within the first 6 years. Sackner's (1966) figures of 34% survival after 5 years and 27% after 7 years are poorer.

The pulmonary lesions are permanent and do not respond to treatment. Patients with renal involvement have a poorer prognosis but can live for years. Medsger *et al.* (1971) noted that all their sixteen patients with renal involvement died within a few months but this has not been my experience. There is no relationship to the presence or type of antinuclear factor. The titres of antinuclear factor did not vary markedly with the clinical course.

The term CRST syndrome should be abandoned as it is not a separate syndrome but only a variant of systemic sclerosis with the same types of systemic involvement and prognosis. The combination of calcinosis, Raynaud's phenomenon, sclerodactyly and telangiectasia occurred in 14% of the series which is rather higher than the 6·3% reported from Warsaw by Szczepański (1972).

The confirmed sex, and possible age, differences and variability of the clinical course underline the importance of age- and sex-matched controls in therapeutic trials in systemic sclerosis. Ideally, patients should be matched for degree of systemic involvement but this is not usually possible. In our experience no drug has yet proved of value in properly conducted clinical trials.

REFERENCES

Bennett, R., Bluestone, R., Holt, P.J.L. & Bywaters, E.G.L. (1971) Survival in scleroderma. *Annals of the Rheumatic Diseases*, 30, 581.

Burch, P.R.J. & Rowell, N.R. (1963) Auto-immunity: aetiological aspects of chronic and systemic discoid lupus erythematosus, systemic sclerosis and Hashimoto's thyroiditis. *Lancet*, ii, 507.

Burch, P.R.J. & Rowell, N.R. (1965) Systemic lupus erythematosus: aetiological aspects. *American Journal of Medicine*, 38, 793.

Kellum, R.E. & Haserick, J.R. (1964) Systemic lupus erythematosus. A statistical evaluation of mortality based on a consecutive series of 299 patients. *Archives of Internal Medicine*, 113, 200.

Medsger, T.A. Jr., Masi, A.T., Rodnan, G.P., Benedek, T.G. & Robinson, H. (1971) Survival with systemic sclerosis (scleroderma). A life-table analysis of clinical and demographic factors in 309 patients. *Annals of Internal Medicine*, 75, 369.

Sackner, M.A. (1966) *Scleroderma*. Grune and Stratton, New York and London.

Szczepański, A. (1972) On the CRST syndrome. *Przeglad Dermatologiczny*, 59, 13.

Tuffanelli, D.L. & Winkelmann, R.K. (1961) Systemic scleroderma. A clinical study of 727 cases. *Archives of Dermatology*, 84, 49.

Tuffanelli, D.L. & Winkelmann, R.K. (1962) Diffuse systemic scleroderma. A comparison with acrosclerosis. *Annals of Internal Medicine*, 57, 198.

Winterbauer, R.H. (1964) Multiple telangiectasia, Raynaud's phenomenon, sclerodactyly, and subcutaneous calcinosis: a syndrome mimicking hereditary hemorrhagic telangiectasia. *Bulletin of Johns Hopkins Hospital*, 114, 361.

British Journal of Dermatology (1976) 95, 61.

An auto-antibody profile in alopecia totalis and diffuse alopecia

REBECCA E.I.COCHRAN*, JOHN THOMSON† AND
RODERICK N.M.MACSWEEN‡

The Department of Dermatology, *The University of Glasgow, The Department of Dermatology,
†The Royal Infirmary, and the Department of Pathology, ‡The Western Infirmary, Glasgow

Accepted for publication 24 October 1975

SUMMARY

Thirty-one patients suffering from alopecia totalis and fourteen patients with idiopathic diffuse alopecia were examined for evidence of an endocrinopathy and their sera screened for organ-specific auto-antibodies. The results were compared with matched control groups of dermatological patients and a random group of dermatological patients. There was no statistically significant difference in the groups and the results were similar to those found in the general population.

Alopecia areata is a common condition accounting for about 2% of new referrals to Dermatology outpatient departments in Britain and America (Ebling & Rook, 1972). Despite this frequency the aetiology remains obscure. Associations between alopecia areata and disease of endocrine glands have been noted (Muller & Winkelmann, 1963; Cunliffe *et al.*, 1968). Several authors have attempted to link alopecia areata with the presence of an increased incidence of circulating auto-antibodies but the results have been varied (Cunliffe *et al.*, 1969; Kern *et al.*, 1973; Main *et al.*, 1975). However, alopecia areata is not a homogeneous entity (Rook, 1975, personal communications) and it is thus difficult to interpret the significance of possible associated features and investigations.

We selected patients with alopecia totalis resulting from alopecia areata. This then represented a particular form of alopecia areata which, since resolution is rare (Ebling & Rook, 1972) can also be considered a more stable and severe form of the disease. We investigated thirty-one patients with alopecia totalis for the presence of circulating auto-antibodies. A small group of patients with diffuse alopecia were investigated for comparison as an entirely different form of alopecia.

MATERIALS AND METHODS

The investigation includes the following groups:

(1) Thirty-one patients with alopecia totalis, twenty-one females whose ages ranged from 13 to 85 years (mean 56·5 years) and ten males aged from 11 to 56 years (mean 28·4 years). The duration of

the total alopecia was from 6 months to 40 years. Two female patients were known to suffer from other diseases; one from pernicious anaemia and one from rheumatoid arthritis.

(3) Fourteen female patients suffering from idiopathic diffuse alopecia. The group had an age range of 44–81 years (mean 65·6 years).

(2 & 4) Forty-five patients suffering from skin disease with, to date, no known association with a disturbed immune mechanism, age and sex matched to the above groups, 1 and 3 respectively. These patients suffered from psoriasis (16), eczema (11), verruca vulgaris (3), basal cell carcinoma (9), basal cell papilloma (2), acne vulgaris (1), stasis ulcers (1), squamous carcinoma (1) and tinea pedis (1).

(5) A further random control group of forty-two patients, aged 7–61 years (mean 32·8 years), twenty-eight female and fourteen male, suffering from skin diseases in which immune mechanisms have not been incriminated: psoriasis (20), verruca vulgaris (5), acne vulgaris (4), rosacea (3), eczema (3), stasis ulcers (3), pityriasis capitis (1), milia (1), paronychia (1) and solar keratoses (1).

In groups 1–4 the patients were examined and any clinical endocrine disorder noted. A sample of blood was obtained from every patient in the five groups and standard indirect immunofluorescent techniques were used to detect antinuclear factor (Beck, 1961), antithyroglobulin antibody (Fulthorpe *et al.*, 1961), thyroid microsomal antibody (Holborow *et al.*, 1959), gastric parietal cell antibody (Adams *et al.*, 1964), antimitochondrial antibody (Goudie, MacSween & Goldberg, 1966), smooth muscle antibody (Johnson, Holborow & Glynn, 1965), adrenal cortex antibody (Goudie *et al.*, 1969), and salivary duct antibody (MacSween *et al.*, 1967). Sera were screened for Rheumatoid Factor using the Rheumaton test (Warner Laboratories) and a quantitative assessment of positive sera was made by the R_3 test (Warner Laboratories).

RESULTS

The positive auto-antibody results are summarized in Table 1. There were no positive results for adrenal cortex antibody. Statistical analysis showed no significant difference in incidence in the groups.

DISCUSSION

The diagnosis of alopecia totalis was made clinically. Scalp biopsies were not performed as this was not considered ethical. The duration of the condition in our series ranged from some months to 40 years. In those who had suffered from the disease for many years it might be considered that the causative pathological process was no longer active and that the continued absence of hair simply represented destruction of the hair follicles. However, this would not seem likely as systemic steroids can stimulate hair regrowth even after many years (Kern *et al.*, 1973).

Failure of hair growth is known to occur in some endocrine disorders but various studies undertaken to demonstrate such an association with alopecia areata have been inconclusive. No definite abnormalities have been established by some authors (Strickler & Greenberg, 1938; Waisman & Kepler, 1941; Walker & Rothman, 1950; Winkelmann, 1958; Salamon, Musafija & Milicevic, 1971; Main *et al.*, 1975). However, thyroid disease associated with alopecia areata was recorded (Muller & Winkelmann, 1963; Morgans, 1964). A raised incidence of circulating organ specific auto-antibodies in vitiligo was reported by Brostoff, Bor & Feiwel (1969) but not confirmed by Woolfson *et al.* (1975). The association between thyroid disease and organ specific auto-antibodies is well established. Since Cunliffe *et al.* (1968) then reported an increased incidence of thyroid disease and vitiligo in patients with alopecia areata it seems reasonable to anticipate an increased incidence of circulating auto-antibodies in alopecia areata.

TABLE 1. Results of auto-antibody tests

	Group 1 Alopecia totalis	Group 2 Matched controls for alopecia totalis	Group 3 Diffuse alopecia	Group 4 Matched controls for diffuse alopecia	Group 5 Random control series	Groups 2, 4 and 5 Totalled control series
No.	31	31	14	14	42	87
Mean age	47·5	47·5	65·6	65·3	32·8	43·2
Range (years)	11–85	9–87	44–81	42–81	7–61	9–87
Sex: male	10	10	0	0	14	24
female	21	21	14	14	28	63
Thyroglobulin Precipitin	3 (9·6)*	0	0	0	0	0
Thyroglobulin TRC titre	1–1/16 2–1/4000 (9·6)	1–1/256 (3·2)	0	1–1/256 (7·1)	0	2 (2·2)
Thyroid microsomal antibody	5 (16·1)	6 (19·3)	3 (21·4)	3 (21·4)	2 (4·7)	11 (12·6)
Gastric parietal cell antibody	7 (22·5)	5 (16·1)	2 (14·2)	3 (21·4)	8 (19)	16 (18·3)
Antinuclear antibody titre	2–1/16 2–1/64H 1–1/64HN 1–1/256H (19·3)	2–1/16 (6·4)	1–1/16 (7·1)	4–1/16 2–1/64H 1–1/256H (50·0)	5–1/16 1–1/64HN 1–1/256HNS (16·6)	16 (18·3)
Rheumatoid factor	7 (22·5)	7 (22·5)	3 (21·4)	4 (28·5)	3 (7·1)	14 (16)
R3 titre	1–1/16 1–1/64 1–1/128 1–1/256	2–1/16	0	1–1/16 1–1/128	1–1/16	0
Mitochondrial antibody	2 (6·4)	0	0	0	2 (4·7)	2 (2·2)
Smooth muscle antibody	2 (6·4)	4 (12·9)	2 (14·2)	5 (35·7)	4 (9·5)	13 (14·9)
Salivary duct antibody	0	0	0	0	1 (2·3)	1 (1·1)

Only significantly positive results are recorded. H, homogeneous pattern; N, nucleolar pattern; S, speckled pattern. * Results expressed as percentages in parentheses.

However, studies to determine whether there is an increase in the incidence of circulating auto-antibodies in alopecia areata have been contradictory (Cunliffe *et al.*, 1969; Kern *et al.*, 1973; Main *et al.*, 1975). In the present series of patients with alopecia totalis, there was no clinical evidence of endocrine disease. In this group one female suffered from pernicious anaemia and another from rheumatoid arthritis and these two patients had the expected associated serological findings. We were unable to find any statistically significant increase in the incidence of circulating auto-antibodies as compared with a matched control series. While our results compare closely with Main *et al.* (1975), we did not find an increased incidence of auto-antibodies to smooth muscle. The differences between the reported series cannot be explained on technical grounds and may reflect the non-homogeneity of alopecia areata. Our group which we consider a definitive type does not conform to the classification by Ikeda (1965).

None of the patients with diffuse alopecia or their controls had evidence of endocrine disease. There was no significant difference in the incidence of auto-antibodies in these two groups or between them and the alopecia totalis groups.

In the control groups we included patients with psoriasis as there has been no substantiation of the suggestion by Cunliffe *et al.* (1969) that there is a negative association between psoriasis and circulating auto-antibodies. None of the psoriatic patients had alopecia.

An additional control group (5) consisting of patients with miscellaneous skin disorders was included as a further comparative group. The incidence of circulating auto-antibodies in this group was similar to that in the other groups. Indeed the incidence in all groups including the combined control group is similar to that in the population in the West of Scotland.

In conclusion, we have been unable to establish any increased incidence of thyroid disease or circulating auto-antibodies in alopecia totalis. While the histological changes and the response to systemic steroids in alopecia areata support the theory of an association with a disturbed immunological mechanism, the aetiology of alopecia areata remains conjectural and the evidence for an immunological pathogenesis minimal.

ACKNOWLEDGMENTS

We are grateful to Mr D.McLaren, the Department of Statistics, The University of Glasgow, for his help and to Mrs Mary Mason for technical assistance.

REFERENCES

Adams, J.F., Glen, A.I.M., Kennedy, E.H., McKenzie, I.L., Morrow, J.M., Anderson, J.R., Gray, K.G. & Middleston, D.G. (1964) The histological and secretory changes in the stomach in patients with auto-immunity to gastric parietal cells. *Lancet*, **i**, 401.

Beck, J.S. (1961) Variations in the morphological patterns of 'auto-immune' nuclear fluorescence. *Lancet*, **i**, 1203.

Brostoff, J., Bor, S. & Feiwel, M. (1969) Auto-antibodies in patients with vitiligo. *Lancet*, **ii**, 177.

Cunliffe, W.J., Hall, R., Newell, D.J. & Stevenson, C.J. (1968) Vitiligo, thyroid disease and auto-immunity. *British Journal of Dermatology*, **80**, 135.

Cunliffe, W.J., Hall, R., Stevenson, C.J. & Weightman, D. (1969) Alopecia areata, thyroid disease and auto-immunity. *British Journal of Dermatology*, **81**, 877.

Ebling, F.J. & Rook, A. (1972) Hair. In: *Textbook of Dermatology* (Ed. by A.Rook, D.S.Wilkinson and F.J. Ebling), 2nd edn, Vol. 2, p. 1598. Blackwell Scientific Publications, Oxford.

Fulthorpe, A.J., Roitt, I.M., Doniach, D. & Couchman, K. (1961) A stable sheep cell preparation for detecting thyroglobulin auto-antibodies and its clinical applications. *Journal of Clinical Pathology*, **14**, 654.

Goudie, R.B., MacSween, R.N.M. & Goldberg, D.M. (1966) Serological and histological diagnosis of primary biliary cirrhosis. *Journal of Clinical Pathology*, **19**, 527.

GOUDIE, R.B., STUART-SMITH, D.A., BOYLE, I.T. & FERGUSON, A. (1969) Serological diagnosis of Addison's disease in patients on prolonged prednisolone therapy for steatorrhoea. *Lancet*, **i**, 186.

HOLBOROW, E.J., BROWN, P.C., ROITT, I.M. & DONIACH, D. (1959) Cytoplasmic localisation of 'complement fixing' auto-antigen in human thyroid epithelium. *British Journal of Experimental Pathology*, **40**, 583.

JOHNSON, G.D., HOLBOROW, E.J. & GLYNN, L.E. (1965) Antibody to smooth muscle in patients with liver disease. *Lancet*, **ii**, 878.

IKEDA, T. (1965) A new classification of alopecia areata. *Dermatologica*, **131**, 421.

KERN, F., HOFFMAN, W.H., HAMBRICK, G.W. & BLIZZARD, R.M. (1973) Alopecia areata. Immunologic studies and treatment with prednisone. *Archives of Dermatology*, **107**, 407.

MACSWEEN, R.N.M., GOUDIE, R.B., ANDERSON, J.R., ARMSTRONG, E., BARR, M., JASANI, M.K., BOYLE, J.A., BUCHANAN, W.W. & WILLIAMSON, J. (1967) Occurrence of antibody to salivary duct epithelium in Sjögren's disease, rheumatoid arthritis and other arthritides. *Annals of the Rheumatic Diseases*, **26**, 402.

MAIN, R.A., ROBBIE, R.B., GRAY, E.S., DONALD, D. & HORNE, C.H.W. (1975) Smooth muscle antibodies and alopecia areata. *British Journal of Dermatology*, **92**, 389.

MORGANS, M.E. (1964) *Hyperthyroidism in the thyroid gland* (Ed. by R.Pitt-Rivers and W.R.Trotter), Vol. **2**, p. 159. Butterworth, London.

MULLER, S.A. & WINKELMANN, R.K. (1963) Alopecia areata. *Archives of Dermatology*, **88**, 290.

SALAMON, T., MUSAFIJA, A. & MILICEVIC, M. (1971) Alopecia areata und Erkrankungen der Thyroidea. *Dermatologica*, **142**, 62.

STRICKLER, A. & GREENBERG, S.I. (1938) Alopecia areata—Roentgen studies of the sella turcica. *Archives of Dermatology and Syphilology*, **37**, 1008.

WAISMAN, M. & KEPLER, E.J. (1941) Alopecia areata: Appraisal of endocrine factors in its causation. *Journal of the American Medical Association*, **116**, 2004.

WALKER, S.A. & ROTHMAN, S. (1950) Alopecia areata: Statistical study and consideration of endocrine influences. *Journal of Investigative Dermatology*, **14**, 403.

WINKELMANN, R.K. (1958) Alopecia areata: Pituitary function assessed by assay of pituitary gonadotrophin in urine. *Journal of Investigative Dermatology*, **30**, 223.

WOOLFSON, H., FINN, O.A., MACKIE, R.M., MCQUEEN, A. & MACSWEEN, R.N.M. (1975) Serum anti-tumour antibodies and auto-antibodies in vitiligo. *British Journal of Dermatology*, **92**, 395.

British Journal of Dermatology (1976) **95**, 67.

Immunological demonstration of multiple esterases in human eccrine sweat

W.P.HERRMANN AND J.HABBIG*

Department of Dermatology, University of Cologne, 5 Köln 41,
Joseph-Stelzmannstrasse 9, Germany

Accepted for publication 29 October 1975

SUMMARY

Eccrine sweat collected from the human skin surface contains at least five different esterases. One of them is a cholinesterase. A non-specific carboxylesterase with the electrophoretic mobility of an α-globulin appears to be a serum protein. Besides this, there are two isoenzymes of human origin migrating with the same electrophoretic mobility as γ-globulins. These two isoenzymes are immunologically identical with a non-specific carboxylesterase occurring in numerous organs and body fluids. Lipase activity could not be demonstrated.

Histochemical investigations have shown that the eccrine sweat gland unit exhibits a strong esterase activity in the secretory tubule as well as in the dermal and epidermal sweat duct (Braun-Falco, 1956; Montagna, Chase & Hamilton, 1951; Steigleder & Löffler, 1956). Immunological studies revealed that eccrine sweat collected from the skin surface contains a non-specific carboxylesterase most probably derived from sebum (Herrman, 1970; Herrman, Heinen & Jung, 1970).

The present paper is concerned with the demonstration of multiple esterases in highly concentrated eccrine sweat. Our studies reveal that the eccrine sweat of man contains at least five ester-splitting enzymes that differ considerably with respect to their enzymatic characteristics.

MATERIALS AND METHODS

Sweat was obtained from thirty-eight males by heat stimulation and concentrated until a protein concentration of 25 mg/ml was reached (= HCSw) (for details see Herrmann & Habbig, 1975). Antisera nos 179 and 180 specifically directed against human sweat proteins (= *RAHCSw*) were prepared by immunization of two rabbits with HCSw as previously described (Herrman & Habbig, 1975). Antibodies directed against serum proteins and against antigens derived from the horny layer, sebum and micro-organisms were removed from RAHCSw by absorption with: (1) Lyophilized human serum proteins (= *RAHCSw-HS*). (2) The content of an epidermal cyst (= *RAHCSw-EC*). (3) Comedones (= *RAHCSw-Co*). (4) Bacterial antigens or organisms isolated from the human skin surface (= RAHCSw-BA I-X).

---

* Present address: 5 Köln 80, Dreikönigshospital Malteserkrankenhaus, Keupstrasse 2–4, Department of Surgery.

Various organisms (*C. acnes*, *S. albus*, *Micrococci*, and *S. aureus*) were cultured for 72 h at 22 and 37°C on a peptone–glucose broth containing yeast extract. Thereafter, the cultures were centrifuged for 20 min at 3000 rev/min. The supernatants were removed and passed through a filter (Seitz K 5) in order to remove bacteria; the sterilized preparations were concentrated by perventilation and finally lyophilized by vacuum evapóration as previously described (Hermann & Herrman, 1968). In addition, RAHCSw was absorbed with lyophilized human seminal plasma (= *RAHCSw-HSP*). Rabbit antiserum no. 452 was prepared by immunization of rabbit with human seminal plasma (= *RAHSP*) as described by Herrman & Schirren (1963). Immuno-electrophoretic analyses were performed according to Grabar & Williams (1955) using the micro-modification of Scheidegger (1955); agar electrophoresis was run under the same conditions. Characterization of enzymes was carried out as previously described (Herrmann, 1972); estimation of lipase activity according to Seligman & Nachlas (1962) using a Zeiss spectrophotometer set (PMQ II), fresh blood serum from a dog serving as a control.

RESULTS

Agar electrophoresis followed by staining with synthetic substrates indicated the presence of 4 ester-splitting fractions, designated I–IV in the sequence of their electrophoretic mobilities. The substrate specificities of the fractions are shown in Table 1.

Immuno-electrophoretic analyses of HCSw with RAHCSw revealed only two esterase-positive precipitation lines (Fig. 1): a major precipitation arc in the region of γ-globulins, corresponding to both fractions III and IV (γ-esterase) and a minor precipitation arc with the electrophoretic mobility of an α-globulin (α-esterase).

The γ-esterase was found to hydrolyze all substrates listed in Table 2. The α-esterase, however, hydrolysed only α-and β-naphthyl acetate, the enzymatic activity being stronger with respect to β-naphthyl acetate than with respect to α-naphthyl acetate. An ester-splitting precipitation line corresponding to fraction I could not be demonstrated.

The immuno-electrophoretic findings are shown in Table 2. These indicate that: (1) The α-esterase is a blood serum protein. (2) The γ-esterase does not occur within epidermal cysts. (3) The γ-esterase of eccrine sweat is immunologically identical with a γ-esterase occurring in comedones as well as in human seminal plasma. (4) Both α-esterase and β-esterase are not bacterial antigens.

TABLE 1. Enzymes occurring in human eccrine sweat as revealed by agar electrophoresis

	Fraction no.			
Substrate	I	II	III	IV
α-Naphthyl acetate	–	+	+	+
β-Naphthyl acetate	(+)	(+)	+	+
Naphthol-AS-acetate	–	–	+	+
Indoxyl acetate	–	–	+	+
Indoxyl + physostigmine	–	–	–	+
Naphthol-AS-Bi-β-D-glucuronide	–	–	(+)	+

+ = strong activity; (+) = weak activity.

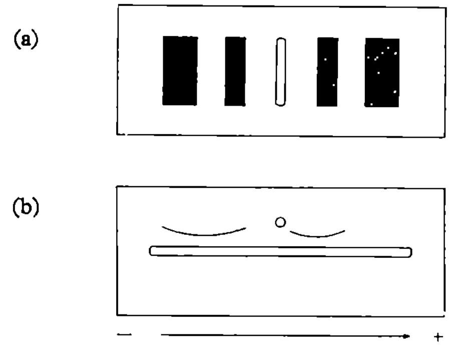


FIGURE 1. Agar electrophoresis (a) of HCSw showing four fractions with esterase activity. Immuno-electrophoretic analysis (b) of HCSw with RAHCSw demonstrates two ester-splitting precipitation lines corresponding to fractions II and III/IV, respectively; substrate: β-naphthyl acetate.

TABLE 2. Immuno-electrophoretic demonstration of esterases in concentrated eccrine sweat by various antisera

	Sweat	
Antiserum	γ-esterase	α-esterase
RAHCSw	+	+
RAHCSw-HS	+	–
RAHCSw-Co	–	+
RAHCSw-EC	+	+
RAHCSw-BA I-X	+	+
RAHCSw-HSP	–	+
RAHSP	+	–

+ = positive; – = negative.

Lipase activity could not be demonstrated in HCSw, although abundant activity was found in the dog serum used as control.

DISCUSSION

Our study shows that the esterases occurring in human eccrine sweat can be separated into four fractions with different electrophoretic mobilities and different enzymatic characteristics. One of these fractions contains both a cholinesterase and a non-specific carboxylesterase hydrolysing various synthetic substrates. Consequently, our sweat preparation contains at least five different esterases.

The non-specific carboxylesterases corresponding to fractions III and IV do not significantly differ with respect to their enzymatic characteristics, except for the fact that the hydrolytic activity of fraction III is stronger than that of fraction IV with respect to naphthol-AS-Bi-β-D-glucuronide. It may well be, therefore, that one of the two fractions (III or IV) contains an additional β-glucuronidase. This, however, remains to be questioned since earlier investigations have shown that several glandular secretions contain a non-specific carboxylesterase that can hydrolyse naphthol-AS-Bi-β-D-glucuronide (Herrmann, 1972). Further experiments are in progress in order to resolve this point.

Our immuno-electrophoretic analyses have shown that the non-specific carboxylesterases migrating with fractions III and IV are immunologically identical. This finding strongly suggests that they are iso-enzymes since their enzymatic characteristics were also found to be similar.

These two iso-enzymes migrating like γ-globulins are not characteristic for eccrine sweat. Earlier investigations revealed that extracts prepared from human epidermis and horny layer, as well as comedones, contain a non-specific carboxylesterase with γ-mobility that is immunologically identical to an esterase occurring in numerous organs and body fluids (Herrmann, 1970).

The absorption experiments presented here demonstrate that the γ-esterases present in our sweat preparation are immunologically identical to that occurring in comedones and other glandular secretions, including human seminal plasma. This finding shows that we are concerned with an enzyme system of widespread distribution throughout the human body. Within epidermal cysts these γ-esterases do not seem to occur. It may be concluded, therefore, that they are not derived from the epidermal epithelium.

The presence of non-specific carboxylesterases with γ-mobility in our sweat preparation suggests that these enzymes are secretion products of the eccrine sweat gland unit. However, we cannot definitely exclude the possibility that they are merely eluted from the superficial sebum film when the sweat runs over the skin surface. In any case, they are not derived from the micro-organisms investigated since both α- and γ-esterase can be precipitated by antisera that have previously been absorbed with bacterial antigens.

The α-esterase corresponding to fraction II was found to be a serum protein. The esterase corresponding to fraction I, however, could not be characterized immunologically because the rabbits immunized with sweat proteins had not produced antibodies against this enzyme fraction.

It is very remarkable that no lipase activity could be demonstrated in our sweat preparation. It may be concluded, therefore, that significant amounts of lipolytic enzymes originating in the microflora of the human skin do not accumulate in eccrine sweat that has been collected from the skin surface.

ACKNOWLEDGMENTS

This study has been supported by the Landesamt für Forschung des Landes Nordrhein-Westfalen. We are indebted to Mr D.Fritsche, M.D. (Department of Bacteriology and Hygiene, University of Cologne) for supplying bacteria and culture media. Professor Dr W.Isselhard (Department of Experimental Surgery, University of Cologne) has kindly supplied the dog's serum. We would like to thank Mrs I.Weyland for technical assistance and Dr R.A.Newman for preparation of the manuscript.

REFERENCES

Braun-Falco, O. (1956) Beitrag zum histochemischen Nachweis von Esterasenin normaler und psoriatischer Haut. *Archiv für klinische und experimentelle Dermatologie*, **202**, 153.

Grabar, P. & Williams, C.A. (1955) Méthode immuno-électrophorétique d'analyse de mélanges de substances antigéniques. *Biochemica Biophysica Acta (Amsterdam)*, **17**, 67.

Hermann, G. & Herrmann, W.P. (1968) Reindarstellung eines niedermolekularen Eiweisskörpers (S_{20W} = 1·1 S) aus der Samenflüssigkeit des Mannes. *Zeitschrift für Naturwissenschaften*, **23b**, 1121.

Herrmann, W.P. (1970) Elektrophoretische und immunologische Untersuchungen an ester-spaltenden Enzymen der menschlichen Haut. *Archiv für klinische und experimentelle Dermatologie*, **236**, 385.

Herrmann, W.P. (1972) Enzymatisch aktive Proteine in menschlichen Spermaplasma. *Beiträge zur gerichtlichen Medizin*, **24**, 175.

Herrmann, W.P. & Habbig, J. (1975) Studies on protein constituents of eccrine sweat. *Archiv für Dermatologische Forschung*, in press.

Herrmann, W.P., Heinen, M. & Jung, K. (1970) Nachweis und Herkunft der unspezifischen Carboxylesterase mit -Mobilität (-Organesterase) an der Oberfläche der menschlichen Haut. *Archiv für klinische und experimentelle Dermatologie*, **238**, 390.

Herrmann, W.P. & Schirren, C. (1963) Immunelektrophoretische Untersuchungen des menschlichen Spermaplasma. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **34**, 134.

Montagna, W., Chase, H.B. & Hamilton, J.B. (1951) Distribution of glycogen and lipids in human skin. *Journal of Investigative Dermatology*, **17**, 147.

Scheidegger, J.J. (1955) Une micro-méthode de l'immuno-electrophorése. *International Archives of Allergy*, **7**, 103.

Seligman, A.M. & Nachlas, M.M. (1962) Lipase. In: *Methoden der enzymatischen Analyse* (Ed. by H.U.Bergmeyer). Verlag Chemie GmbH, Weinheim/Bergstrasse.

Steigleder, G.K. & Löffler, H. (1956) Zum histochemischen Nachweis unspezifischer Esterasen und Lipasen. *Archiv für klinische und experimentelle Dermatologie*, **203**, 41.

British Journal of Dermatology (1976) 95, 71.

Pharmacology and Treatment

Septrin in the treatment of dermatitis cruris pustulosa et atrophicans

WITOLD K. JACYK

Department of Medicine, Ahmadu Bello University Hospital, Kaduna, Nigeria

Accepted for publication 9 October 1975

SUMMARY

The results of treatment of 30 Nigerian patients suffering from dermatitis cruris pustulosa et atrophicans by a 7-week course of Septrin are described. Septrin was effective in twenty-three of twenty-four patients who completed the study. The best therapeutic effects were observed in the early, pustular phase of the disease.

Dermatitis cruris pustulosa et atrophicans (DCPA), first described by Clarke (1952) in Lagos, is characterized by a protracted course of eruptions with follicular pustules, cutaneous oedema, scaling, atrophy and a remarkable shininess of the skin of both legs between the knee and ankle. An excellent clinical description of the condition was given by Harman (1968). Sugathan, Zacariah & Joy (1973) proposed the term 'Folliculitis cruris pustulosa et atrophicans' and distinguished four grades in the course of the condition finding a significant relation between the grade of the illness and its duration: Grade I—follicular pustules only. Grade II—follicular pustules are predominant, but there is also a significant number of infiltrated papules, some of which show excoriations, crusting and delicate scaling. Grade III—a number of infiltrated scaly papules, atrophy, shininess, marked alopecia. Pustules are few or absent. Grade IV—atrophic shiny scaly skin with almost complete alopecia, few papules and no pustules.

The incidence of DCPA in some geographical regions is fairly high. Shrank & Harman (1966) found it in 2·2% of patients attending the skin clinic in Ibadan, Nigeria. According to Thambiah (1967, personal communication, cited by Harman) 3–4% of dermatological out-patients in Madras are suffering from DCPA. In the skin clinic in Kaduna, Northern Nigeria, DCPA constituted 4·8% of all out-patients seen in the year 1974.

Spontaneous resolution of the lesions seems probable as no patients over 30 years of age have been seen by Harman (1968), and in a large group of patients studied by Sugathan *et al.* (1973) the majority belonged to the 16–31 age group. Fungal infections have been ruled out. Cultures of material collected from the pustules indicate a bacterial origin. In all of the thirteen cases of Harman (1968) and in seventy-nine patients of Sugathan *et al.* (1973) cultures yielded staphylococci. The condition is characterized by its stubborn resistance to treatment, although Harman (1968) noticed in some cases a good response to oral tetracycline.

Septrin (Trimethroprim/Sulphamethoxazole) had a good effect upon our three first cases of DCPA and we felt it interesting to investigate its effect in a group of patients with the condition.

MATERIALS AND METHODS

Thirty consecutive Nigerian out-patients who satisfied Harman's clinical criteria for the diagnosis of DCPA were the subjects of this study. Three patients were found to have grade I, twenty-two grade II and five grade III. None had grade IV. Six patients had an involvement of other than the classical sites—the thighs and forearms. The patients represented various tribes and occupations and there were 16 males and 14 females. Ages of the patients at the time of the study were from 14 to 27 years. Symptoms of the disease had lasted from 3 months to 4 years, the majority 12–20 months. Only eight patients had been treated previously with various topical applications and/or with penicillin injections.

Treatment with Septrin was for 7 weeks as follows: 1st week—two tablets twice daily; 2nd week—one tablet three times daily; 3rd week—one tablet twice daily; and then one tablet daily for a further 4 weeks. The patients were seen weekly when taking the tablets and then every 3–4 weeks for a further 6–9 months. No topical or other systemic antibacterial therapy was administered throughout the treatment and follow-up period. The therapeutic results were evaluated as: + + + response indicating complete resolution of lesions; + + response indicating that although pustules were no longer present, a few scattered infiltrated papules still remained; + response indicating that the number of pustules considerably diminished but there was no significant effect upon other types of lesions—papules, crusts and scales; 0 response—no effect.

RESULTS

Of the thirty patients treated, twenty-four completed the study. Five patients stopped attending the clinic and in one patient side effects caused the cessation of the therapy. Table 1 gives the results of treatment. Septrin was effective in twenty-three cases, ineffective in one case. A significant relationship was found between the rate of response and the clinical features. The best therapeutic response was observed in those cases in which pustular folliculitis dominated. In nineteen cases there was a complete disappearance of pustules and a follow-up of at least 6 months revealed no recurrence. Patients with a predominantly papular eruption also reacted favourably. Although complete resolution of infiltrated papules was not usually seen, the administration of Septrin resulted in significant diminution of these lesions and prevented the development of scaling and atrophy. The time needed for

TABLE 1. Results of the treatment of dermatitis cruris pustulosa et atrophicans with Septrin

Grade	Response			
	+++	++	+	0
I	3	—	—	—
II	2	12	3	—
III	—	2	1	1

evident amelioration of the condition varied from case to case. In patients with grade I the lesions disappeared in 2 weeks, subjects with grade II responded usually within 3–4 weeks, but in the first two cases withdrawal of treatment resulted in new crops of pustules. Subsequent lengthening of the treatment period for a further 3–4 weeks appeared to keep the lesions under control. All subsequent patients were treated for 7 weeks. In the longstanding cases of DCPA characterized by evident atrophy and alopecia the response was less favourable. No relationship was found between the rate of response and the age or sex of the patients. In one patient nausea and vomiting caused the withdrawal of the drug.

DISCUSSION

It is too early to say that Septrin will cure patients with DCPA but it is evident that the drug has an effect upon the condition, particularly upon its initial pustular phase. Individual variability of the course of the disease is known but no adequate information is available on the changes occurring with time. Therefore, a period of 6–9 months observation after treatment is not long enough to state that Septrin prevents the appearance of the later developing features, i.e. scaling, atrophy and loss of hair, and a longer follow-up is required. Nevertheless as this disorder is extremely difficult to treat, the results obtained with Septrin seem to be worthy of notice. Tolerance of the drug was good. There are several reports in the literature of good tolerance to similar long-term treatment of acne with Septrin (Strauss & Pochi, 1970; Cotterill *et al.*, 1971; Hersle, 1972).

ACKNOWLEDGMENT

Thanks are due to Professor E.H.O.Parry (ABU Zaria) for his advice and encouragement.

REFERENCES

Clarke, G.H.V. (1952) A note on dermatitis cruris pustulosa et atrophicans. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **46**, 558.

Cotterill, J.A., Cunliffe, W.J., Forster, R.A., Williamson, D.M. & Bulusu, L. (1971) A comparison of trimethoprimsulphamethoxazole with oxytetracycline in acne vulgaris. *British Journal of Dermatology*, **84**, 366.

Harman, R.R.M. (1968) Dermatitis cruris pustulosa et atrophicans, the Nigerian shin disease. *British Journal of Dermatology*, **80**, 97.

Hersle, K. (1972) Trimethoprim-sulphamethoxazole in acne vulgaris. A double blind study. *Dermatologica*, **145**, 187.

Shrank, A.B. & Harman, R.R.M. (1966) The incidence of skin diseases in a Nigerian teaching hospital dermatological clinic. *British Journal of Dermatology*, **78**, 235.

Strauss, J.S. & Pochi, P.E. (1970) The effect of sulfisoxazole-trimethoprim combination on titratable acidity of human sebum. *British Journal of Dermatology*, **82**, 493.

Sugathan, P., Zacariah, J. & Joy, M.I. (1973) Folliculitis cruris pustulosa et atrophicans. *Indian Journal of Dermatology and Venereology*, **39**, 35.

British Journal of Dermatology (1976) 95, 75.

The treatment of psoriatic nail dystrophy with intradermal steroid injections

R.D.G.PEACHEY, R.J.PYE AND R.R.M.HARMAN

Department of Dermatology, Bristol Royal Infirmary, Bristol BS2 8HW

Accepted for publication 9 October 1975

SUMMARY

Thirty-seven patients with psoriatic nail dystrophy were treated with intradermal nail-fold injections of either triamcinolone acetonide or triamcinolone hexacetonide, using the Port-O-Jet needleless injector.

Mild nail-matrix changes showed a very good response to treatment, whilst nail-bed and/or hyponychial changes causing onycholysis and the more severe types of dystrophy usually showed little improvement.

Although in occasional cases this may be a useful form of treatment, we would not recommend its general use in patients with psoriatic nail dystrophy.

It is generally held that the primate nail is formed from the nail matrix, which is that part of the nail bed extending from the proximal part of the nail fold to the lunula. The upper surface of the nail is formed from the most proximal part of the matrix, while the deeper layers are formed from the more distal part. There is some dispute over the part played by the nail-bed distal to the lunula. Zaias & Avarez (1968) believed that this plays no part in nail formation, and only provides support for the nail plate, while Lewis (1954) considered that it makes a small contribution to the formation of the under surface of the nail plate.

Zaias (1969) correlated the various types of nail abnormality seen in psoriasis with psoriatic involvement of the various nail components. In his view psoriatic changes in the nail matrix give rise to nail-plate abnormalities, pits and grooves, while abnormalities in the nail bed give rise to discoloured areas beneath the nail, and to subungual keratosis. In addition he believed that changes may occur in the hyponychium (the most distal part of the nail bed and adjacent skin beneath the free edge of the nail) which may lead to onycholysis and subungual keratosis. Nail-bed and hyponychial changes often occur together leading to extensive onycholysis and subungual keratosis.

Gerstein (1962) first showed that fluorinated corticosteroids, injected by needle into the posterior nail fold, produced improvement in some dystrophic nails in patients with psoriasis and lichen planus. In 1972 Abell reported a small series of patients with psoriatic nail dystrophy treated with triamcinolone acetonide injections, using the Port-O-Jet needleless injector, and observed a remission in nearly

80% of treated nails. In a further report Abell & Samman (1973) found that dystrophies involving the nail matrix tended to improve markedly, but that onycholysis was frequently unaffected.

The present survey was carried out to assess further the usefulness of this form of treatment in patients with psoriatic nail dystrophy.

SUBJECTS AND METHODS

Patients with psoriasis who complained of associated nail dystrophy were referred to a special clinic. Using the Port-O-Jet needleless injector as described by Abell & Samman (1973), one to three deep intradermal injections of 0.1 ml of either triamcinolone acetonide (5 mg/ml) or triamcinolone hexacetonide (5 mg/ml) were given into the posterior nail fold of affected nails. The Port-O-Jet injector was sterilized prior to use with 1% chlorhexidine in 70% industrial spirit, and then flushed through with sterile normal saline. Nail changes were assessed clinically as (a) matrix changes, (b) nail-bed and/or hyponychial changes or (c) a mixture of both groups. All patients with nail-bed and/or hyponychial changes had onycholysis and most, in addition, had some discoloration beneath the nail plate.

Standardized photographs of the nails of both hands were taken before and after a course of treatment. Progress was assessed clinically at each attendance, and charted.

The results in each patient were reviewed 4 weeks after the end of a course of treatment, and scored as follows: no change; + slight improvement; + + marked improvement; + + + normal or nearly normal nail.

RESULTS

Group 1. Eighteen patients (10 male and 8 female) with psoriatic nail dystrophy were treated with injections of 0.1 ml triamcinolone acetonide (5 mg/ml) on three separate occasions at intervals of 4–6 weeks. The results of treatment are shown in Table 1. Of ten patients with matrix changes, two showed no change and eight showed improvement. Nail-bed and/or hyponychial changes showed no change in thirteen of seventeen patients, and were improved in four—of whom only one had marked improvement.

Group 2. In view of the generally poor results in Group 1, particularly in patients with nail-bed and/or hyponychial changes, a further group of ten patients (3 male and 7 female) were treated in the same way as Group 1 but at shorter intervals. It was intended that injections should be given at intervals of 2 weeks, but owing to recurrent mechanical problems with the Port-O-Jet apparatus some intervals were prolonged to 4 weeks. The number of treatments given to individual patients varied from 3 to 9. Three patients developed small areas of atrophy in the nail folds at the site of the steroid injections. The results of treatment are summarized in Table 1. Four patients had matrix changes and all showed some improvement. Nine patients had nail-bed and/or hyponychial changes, and of these eight showed no change, while one showed a return to normal.

Group 3. A group of nine patients (5 male and 4 female) were treated on three or four occasions as before but using 0·1 ml triamcinolone hexacetonide (5 mg/ml) at intervals of 5–7 weeks. This preparation has a more prolonged depot action than triamcinolone acetonide, and it was hoped that this would allow less frequent injections to be given. Three patients complained of considerable pain starting 15–30 minutes after the injections and lasting for several hours. One patient developed areas of nail-fold atrophy. Six patients with matrix changes improved with treatment (Table 1), but nail-bed and/or hyponychial changes—present in five patients—were unchanged in two and only slightly improved in three.

TABLE 1. Results of nail-fold injections with triamcinolone in three groups of patients with psoriatic nail dystrophy

		No. of patients improved, degree of improvement and type of nail dystrophy		
Treatment	No. of cases	Matrix changes only	Nail-bed and/or hyponychial changes only	Matrix changes and nail-bed and/or hyponychial changes
Group 1	18	1	8	9
(triamcinolone acetonide)	(10 M, 8 F)	1 (+ + +)	5 (no change) 2 (+) 1 (+ +)	Matrix changes 2 (no change) 3 (+) 4 (+ +) Nail-bed and/or hyponychial changes 8 (no change) 1 (+)
Group 2	10	1	6	3
(triamcinolone acetonide)	(3 M, 7 F)	1 (+ +)	5 (no change) 1 (+ + +)	Matrix changes 2 (+) 1 (+ +) Nail-bed and/or hyponychial changes 3 (no change)
Group 3	9	4	3	2
(triamcinolone hexacetonide)	(5 M, 4 F)	1 (+) 2 (+ +) 1 (+ + +)	1 (no change) 2 (+)	Matrix changes 1 (+) 1 (+ +) Nail-bed and/or hyponychial changes 1 (no change) 1 (+)

DISCUSSION

We had hoped to compare the three regimes in the treatment of psoriatic nail dystrophy, but our poor results and persistent mechanical troubles with the Port-O-Jet injector made us end the trial prematurely. The small number of patients in each group precludes a valid comparison of the treatment regimes, but the pooled data from all three groups (Table 2) shows that pitting, ridging and other nail-matrix changes were improved in 90% of patients, while nail-bed and/or hyponychial changes (mainly onycholysis) improved in only 26%.

Abell & Samman (1973) using triamcinolone acetonide reported improvement in 91% of patients with nail-matrix changes in psoriasis, and in 62% of patients with onycholytic changes. Bleeker (1975) noted 'primary cure or improvement' in 90% of psoriatic nails with matrix changes and 49% of those with onycholysis.

Although a long-term follow-up of our cases has not been carried out, it is clear, from the work of Abell & Samman (1973) and Bleeker (1975), that relapse of both matrix changes and onycholysis is common in patients who are observed for some months following successful treatment.

The Port-O-Jet injector is an expensive piece of apparatus and treatment is time-consuming for the doctor, uncomfortable for the patient, and necessitates frequent attendance at a special dermatology clinic.

In our experience mild and relatively trivial matrix changes usually show a good response to treat-

TABLE 2. Over-all results of nail-fold injections with triamcinolone in thirty-seven patients with psoriatic nail dystrophy

Matrix changes	Nail-bed and/or hyponychial changes
20	31
2 (no change)	23 (no change)
7 (+)	6 (+)
9 (++)	1 (++)
2 (+++)	1 (+++)

ment. This might be expected as nail-fold injections bring the triamcinolone into close proximity to the affected proximal and upper part of the nail matrix. On the other hand, it is not surprising that nail-bed and hyponychial changes (such as onycholysis and discolouration) do so badly as steroid injected into the nail fold is deposited a long way from the site of the abnormality. Most of our patients with really troublesome and distressing nail dystrophy had nail-bed and/or hyponychial changes, and it is unfortunate that this is the group that is least helped by intradermal nail-fold steroid injections. Whilst in very occasional patients this may be a useful form of treatment we would not recommend its general use in patients with psoriatic nail dystrophy.

ACKNOWLEDGMENTS

Our thanks are due to the South Western Regional Health Authority who financed this investigation out of Research Funds, and to Lederle Laboratories who provided us with supplies of triamcinolone hexacetonide.

REFERENCES

ABELL, E. (1972) Treatment of psoriatic nail dystrophy. *British Journal of Dermatology*, **86**, 79.

ABELL, E. & SAMMAN, P.D. (1973) Intradermal triamcinolone treatment of nail dystrophies. *British Journal of Dermatology*, **89**, 191.

BLEEKER, J.J. (1975) Intradermal triamcinolone acetonide treatment of psoriatic nail dystrophy with Port-O-Jet. *British Journal of Dermatology*, **92**, 479.

GERSTEIN, W. (1962) Psoriasis and lichen planus of nails. *Archives of Dermatology*, **86**, 419.

LEWIS, B.L. (1954) Microscopic studies of fetal and mature nail and surrounding soft tissue. *Archives of Dermatology and Syphilology*, **70**, 732.

ZAIAS, N. & ALVAREZ, J. (1968) The formation of the primate nail plate: an autoradiographic study in the squirrel monkey. *Journal of Investigative Dermatology*, **51**, 120.

ZAIAS, N. (1969) Psoriasis of the nail: a clinico-pathologic study. *Archives of Dermatology*, **99**, 567.

British Journal of Dermatology (1976) **95**, 79.

High dose dapsone toxicity

WALTER B.SHELLEY AND MANFRED I.GOLDWEIN

Departments of Dermatology and Medicine, University of Pennsylvania School of Medicine,
Philadelphia, Pennsylvania

Accepted for publication 3 November 1975

SUMMARY

A description of the clinical and biochemical course is given for a patient who developed haemolysis and methaemoglobinaemia following dapsone therapy in high dosage for 2 weeks.

Dapsone has long intrigued pharmacologists because of its ability to induce red cell haemolysis, regularly in normal individuals. The phenomenon is dose related, appearing even with the small otherwise well-tolerated therapeutic dosage of 25–100 mg per day. Under these circumstances, compensatory mechanisms permit asymptomatic long-term therapy. In contrast, high dosage dapsone rapidly induces dramatic life threatening haematological changes. As an example of this, we wish to report our experiences with a woman admitted to the hospital as a result of having taken dapsone in a dose averaging 600 mg/day for 14 days.

CASE REPORT

This 68-year-old white woman was admitted to the University Hospital on an emergency basis. Appearing pale, cyanotic and jaundiced, she was dyspnoeic, febrile, lethargic and too weak to walk. Her skin was covered with excoriations and haemorrhagic crusted lesions. A history revealed that for the past 6 weeks she had had a markedly pruritic generalized vesiculo-bullous eruption which had been diagnosed as dermatitis herpetiformis. A biopsy had confirmed this diagnosis histologically, and had ruled out pemphigus and pemphigoid. Because of the severity of her skin lesions, her local dermatologist had prescribed dapsone (4,4′-diaminodiphenyl sulfone; DDS) in a dosage of 400 mg a day for 1 week, increasing it to 800 mg a day for the week just prior to admission. This had ameliorated the cutaneous problem, but in the days prior to admission she lost all appetite, became ill and virtually collapsed. She had previously always been in good health, neither she nor her family requiring medical attention.

Examination further disclosed a blood pressure of 150/80, pulse 112, temperature 102°F, respirations 36, but general physical examination was not noteworthy except for the skin changes described already, as well as peripheral cyanosis.

On admission the haemoglobin level was 10·7 g%, the haematocrit 32%. The reticulocyte count was 25%. A blood smear showed numerous nucleated red blood cells, Howell-Jolly bodies, spherocytes, helmet cells and fragmented red cells. Heinz bodies were not seen. The white blood cell count was 14,600, with a differential of 50% polymorphonuclears, 10% band forms, 11% lymphocytes, 8% monocytes, 4% plasma cells and 17% eosinophils. The nucleated red cell count was 13,800 per mm^3. The platelet count was 200,000 per mm^3. The total bilirubin level was 2·08 mg/dl, 1·0 mg/dl of which was conjugated. The arterial blood revealed a pH of 7·51, Po_2 66 mm, Pco_2 29 mm and HCO_3 23·2 meq/l. The methaemoglobin level was 1·7 g/dl, and the dapsone concentration was 5·3 mg/dl. Repeated electrocardiograms were normal.

Daily monitoring for the subsequent 3 weeks showed a steady fall in the haemoglobin level to 6·3 g% on the 4th day. Following administration of 3 units of packed red blood cells the level rose to 11·8 g where it essentially remained. The reticulocyte count climbed to 37% by the 3rd day, then tapered off, falling below 4% by the 10th day. The eosinophilia disappeared after 1 week with levels returning to below 5%.

Serum electrolytes, glucose, blood urea, creatinine, calcium, uric acid, transaminases, and alkaline phosphatase levels showed no significant change. Serum protein electrophoretic studies were normal.

The Coomb's test was negative, and a G6PD level was normal. Serum immunofluorescent studies for anti-epithelial antibodies were negative.

Complete studies for an underlying malignancy were negative. These included chest X-ray, upper and lower gastrointestinal series, urogram, proctoscopic, pelvic examination with Pap smear and a CEA determination.

Elimination of dapsone, intravenous fluids and diuretics as well as the packed red cell transfusions were the mainstay of therapy. Within 3 days the cyanosis was not noticeable. By the 5th day the serum dapsone had fallen to 0·09 mg/dl and the vesiculo-bullous lesions began to reappear. These were controlled by high dosage prednisone therapy (80 mg/day). This in turn was gradually tapered and essentially complete control for the subsequent months was maintained by a daily dose as low as 5 mg every 3rd day, coupled with a gluten-free diet.

Although the patient complained of numbness of her hands and feet, the major complication was the development of a band of sharply demarcated gangrene on the distal phalanx of the right great toe which suddenly appeared on the 10th day (Fig. 1). Conservative treatment was followed by ulceration and complete healing 5 months later.

One year later, the patient died as a result of a mycocardial infarct. An autopsy revealed no systemic disease.

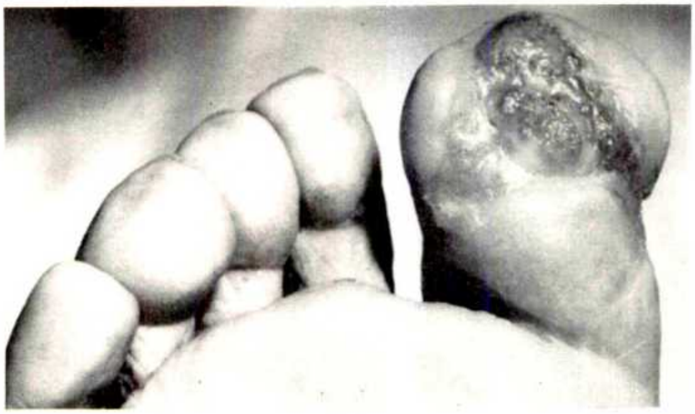


FIGURE 1. Ulcer of great toe following dapsone-induced gangrene. This lesion completely healed without surgical intervention.

DISCUSSION

Dapsone, 4,4′-diaminodiphenyl sulfone, not only is the drug of choice for the treatment of leprosy (Ellard *et al.*, 1973), but has a role to play in the prophylaxis of malaria. It also has a remarkably specific effect in suppressing the blistering lesions of dermatitis herpetiformis. It is absorbed unchanged into the blood stream where it circulates as free dapsone. Much of it is excreted by the kidneys within 24 h, and over 70% is usually removed from the body within 5 days (Alexander *et al.*, 1970). It is usually prescribed in an oral dose of 100–200 mg per day, the serum level being 0·2–0·4 mg%.

Even at low therapeutic levels, it regularly causes haemolysis (Degowin *et al.*, 1966). The degree of haemolysis is clearly dose related. Thus at dosage levels of 50 mg daily, reticulocytosis and minimal shortening of the red cell life-span occur, yet the haemoglobin levels remain normal due to compensatory marrow hyperplasia. At higher dosage levels anaemia becomes evident due to the more severe degrees of haemolysis. Haemolysis is probably due to intracellular oxidant activity of the drug (Rasbridge & Scott, 1973). The precise mechanism may involve lipid peroxidation and reduction of sulphydryl group activity in the red cell membrane (Rasbridge & Scott, 1973). This would also explain the occurrence of more severe haemolysis in persons with G6PD deficiency (Degowin *et al.*, 1966). At dosage levels of 100 mg and above, fragmented red cells are regularly seen; the percentage of fragmented cells is also dose related (Cream & Scott, 1970). The cause of this phenomenon is unclear; it does not appear to be related to microangiopathy or to mechanical trauma.

Dapsone therapy is likewise accompanied by methaemoglobinaemia, though usually to a degree of less than 5%. It is this methaemoglobinaemia which accounts for the cyanosis. Recent studies would suggest that the active responsible compound is not dapsone itself, but a monohydroxylamine metabolite formed by hepatic microsomes (Israili *et al.*, 1973).

Both the haemolysis and the methaemoglobinaemia sharply reduce the oxygen content and oxygen-carrying capacity of the blood. This in turn can lead to disastrous tissue hypoxia (Cream & Scott, 1970), tachypnoea, lethargy, all the signs presented by our patient initially. Our patient, however, showed no evidence of coronary insufficiency which may be seen at times.

Although dapsone can cause all of the immune and idiosyncratic drug rashes, such as a morbilliform eruption, erythema multiforme, lupus erythematosus, and toxic epidermal necrolysis (Millikan & Harrell, 1970), the history here made it apparent that the eruption antedated the dapsone therapy and was indeed dermatitis herpetiformis.

The presenting problem reflected the toxic effect of high dose dapsone on the haematological system. This has but rarely been documented in the literature (Hedstrand, 1968), in as severe a form as seen in our patient, although dramatic, single, acute, accidental overdosage has been recorded in children (Schvartsman, Borges & Mello, 1966).

Emphasis has been placed on the response of the erythrocyte to dapsone, but it must be recognized that in a few idiosyncratic patients, agranulocytosis as well as leukaemoid drug reactions may develop (Graham, 1975). Finally, an ill-defined peripheral neuropathy may occur and could have played a role in our patient's symptoms (duVivier & Fowler, 1974).

Once the cause is recognized, and the drug interdicted, the central themes for therapy are the promotion of excretion of the residual dapsone by diuresis, and the replacement of red cells by packed cell transfusion. Treatment by dialysis is not generally regarded as efficient and the use of methylene blue, classically cited, may produce an exacerbation of the haemolytic process, as a consequence of its coupled oxidation of reduced NADPH, thereby impairing any necessary reduction of oxidized glutathione (Goldstein, 1974).

REFERENCES

ALEXANDER, J.O.D., YOUNG, E., MCFADYEN, T., FRASER, N.G., DUGUID, W.P. & MEREDITH, E.M. (1970) Absorption and excretion of ^{35}S dapsone in dermatitis herpetiformis. *British Journal of Dermatology*, **83**, 620.

CREAM, J.J. & SCOTT, G.L. (1970) Anaemia in dermatitis herpetiformis. The role of dapsone-induced haemolysis and malabsorption. *British Journal of Dermatology*, **82**, 333.

DU VIVIER, A. & FOWLER, T. (1974) Possible dapsone-induced peripheral neuropathy in dermatitis herpetiformis. *Proceedings of Royal Society of Medicine*, **67**, 439.

DEGOWIN, R.L., EPPES, R.B., POWELL, R.D. & CARSON, P.E. (1966) The haemolytic effects of diaphenylsulfone (DDS) in normal subjects and in those with glucose-6-phosphate-dehydrogenase deficiency. *Bulletin of World Health Organization*, **35**, 165.

ELLARD, G.A., GAMMON, P.T., HELMY, H.S. & REES, R.J.W. (1973) Urine tests to monitor the self-administration of dapsone by leprosy patients. *American Journal of Tropical Medicine and Hygiene*, **23**, 464.

GOLDSTEIN, B.D. (1974) Exacerbation of dapsone-induced Heinz body hemolytic anemia following treatment with methylene blue. *American Journal of Medicine Science*, **267**, 291.

GRAHAM, W.R.JR (1975) Adverse effects of dapsone. *International Journal of Dermatology*, **14**, 494.

HEDSTRAND, H. (1968) A case of acute side-reactions to diaminodiphenylsulphone (DDS). *Acta dermato-venereologica*, **48**, 528.

ISRAILI, Z.H., CUCINELL, S.A., VAUGHT, J., DAVIS, E., LESSER, J.M. & DAYTON, P.G. (1973) Studies of the metabolism of dapsone in man and experimental animals: formation of n-hydroxy metabolites. *Journal of Pharmacology and Experimental Therapeutics*, **187**, 138.

MILLIKAN, L.E. & HARRELL, E.R. (1970) Drug reactions to the sulfones. *Archives of Dermatology*, **102**, 220.

RASBRIDGE, M.R. & SCOTT, G.L. (1973) The haemolytic action of dapsone: the effect on red-cell glycolysis. *British Journal of Haematology*, **24**, 169.

RASBRIDGE, M.R. & SCOTT, G.L. (1973) The haemolytic action of dapsone: changes in the red-cell membrane. *British Journal of Haematology*, **24**, 183.

SCHVARTSMAN, S., BORGES, M.A.G. & MELLO, P. (1966) Alteracoes Hematologicas nas Intoxicacoes Acidentois Agudas Por Sulfonas. *Revista do Hospital das Clinicas; Faculdade de Medicina, Universidade de São Paulo*, **21**, 42.

British Journal of Dermatology (1976) 95, 83.

Ciclopirox: a new topical pyrodonium antimycotic agent

A DOUBLE-BLIND STUDY IN SUPERFICIAL DERMATOMYCOSES

V.N.SEHGAL

Department of Venereology and Dermatology, Goa Medical College and Hospital,
Panaji-403001, Goa, India

Accepted for publication 3 November 1975

SUMMARY

One percent Ciclopirox solution, a new topical antimycotic agent, was used in a double-blind controlled trial in 105 dermatomycosis patients. Twelve of the patients were lost to follow-up. Of the remaining ninety-three patients, forty-four were treated with the active drug, forty-nine with a placebo. The cure rate amongst those using ciclopirox was significantly superior to that of the placebo. The drug was effective against *Trichophyton rubrum*, *T. violaceum*, *T. mentagrophytes* and *Malassezia furfur*.

Ciclopirox (HOE 296)* is 1-hydroxy-4-methyl-6-cyclohexyl-2-(1H)-pyridone ethanolamine salt. Its structure is shown in Fig. 1. *In vitro* studies have shown that Ciclopirox is effective against fungi responsible for infections of the skin, such as dermatophytes and yeasts. The minimum inhibitory concentration against these fungi varied from 0·98 to 3·9 μg/ml. *In vivo* studies showed it was also effective in the treatment of dermatophytosis of guinea-pigs (Romiti, 1974). The compound also has an antibacterial action against a variety of Gram-positive and Gram-negative organisms. Moreover, Ciclopirox was found to be relatively free from side-effects and toxicity.

In view of this, a double-blind clinical study was undertaken to compare Ciclopirox with a placebo in superficial dermatomycoses.

MATERIALS AND METHODS

One hundred and five patients of either sex above the age of 18 years were admitted to the study. Only those patients were included in whom the area of involvement did not exceed 10% of the body surface and who had not received any specific anti-mycotic treatment orally. Pregnant women and patients with secondarily infected lesions were not included in the study.

Diagnosis of the fungal lesion was confirmed by a microscopic examination of scrapings cleared by 10% potassium hydroxide and by culture of scrapings on Sabouraud's dextrose agar supplemented with penicillin, streptomycin and actidione.

---

* Hoechst Pharmaceuticals Limited, Bombay-400 020.

Every patient admitted to the study was assigned a serial number and as per the randomization table received either Ciclopirox 1% solution in polyethylene glycol 400 or the placebo solution containing the vehicle only. The patients were instructed to apply gently a few drops of the solution to cover the lesions and an area of 2 cm around. The medication was applied twice daily for 4 weeks. No other topical or systemic medication was used throughout the trial.

Each patient was clinically examined daily for the first 5 days. Thereafter, both mycological and clinical examinations were carried out on the 5th, 7th, 14th, 21st and 28th days of treatment. Treatment was discontinued if there was no improvement within 2 weeks or cure was obtained during this period.

To evaluate safety, the following investigations were undertaken before commencement of treatment and repeated at the end of the therapy: Haemoglobin, WBC, SGOT, SGPT, serum bilirubin, prothrombin time, blood urea and routine urine examination

During the follow-up period, twelve patients failed to report and were considered drop-outs. Thus ninety-three cases were finally available for analysis of which forty-four were on the active drug and forty-nine the placebo.

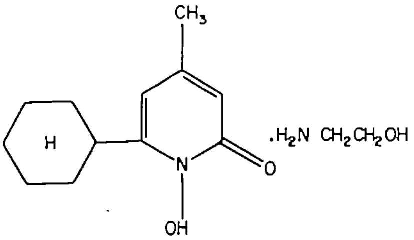


FIGURE 1.

TABLE 1. Numbers of cases showing complete or near complete regression according to clinical and mycological diagnosis

Clinical diagnosis	Mycological diagnosis	Ciclopirox	Placebo
Tinea corporis	*T. rubrum*	5/8	1/4
	T. violaceum	4/4	0
	T. mentagrophytes	1/2	1/2
	Negative culture	1/1	1/2
Tinea cruris	*T. rubrum*	4/10	5/15
	T. violaceum	1/2	2/5
	T. mentagrophytes	1/2	0/3
	T. verrucosum	1/1	0
	Negative culture	3/8	1/4
Tinea corporis and *cruris*	*T. rubrum*	0/1	0/5
	T. violaceum	0	0/2
	Negative culture	0/1	1/3
T. pedis	*T. rubrum*	1/1	0/1
Pityriasis versicolor	*Malasezzia furfur*	3/3	2/3
Total		25/44	14/49
		($P<0{\cdot}02$)	

TABLE 2. Results of patients who had positive culture and presence of fungi on microscopic examination (KOH)

Day of follow-up	No. of cases which became negative subsequently			
	Microscopic (KOH)		Culture	
	Ciclopirox	Placebo	Ciclopirox	Placebo
Pretreatment	0/44	0/49	0/30	0/36
7	22/44	9/49	—	—
	$(P<0{\cdot}01)$			
14	25/43	13/48	13/27	6/33
	$(P<0{\cdot}01)$		$(P<0{\cdot}05)$	
21	24/39	16/47	14/24	9/34
	$(P<0{\cdot}02)$		$(P<0{\cdot}05)$	
28	23/37	15/42	13/24	9/31
	$(P<0{\cdot}05)$		(N.S.)	

N.S. = not significant.

RESULTS

Complete or near complete regression was defined as absence of fungi on microscopic examination, negative culture and marked to complete relief from symptoms. Results are shown in Table 1. It will be observed that twenty-five of fourty-four patients receiving Ciclopirox were considered cured while fourteen of the forty-nine cases receiving placebo medication showed similar improvement $(P<0{\cdot}02)$.

Table 2 shows the analysis of the microscopic and cultural results. It will be observed that a significantly larger number of patients receiving Ciclopirox became microscopically negative on days 5,

TABLE 3. Percentage of patients showing disappearance of symptoms or marked improvement by clinical evaluation with negative microscopic examination on different days of follow-up

Day of follow-up	% of patients	
	Ciclopirox	Placebo
7	10/44 (22·7)*	4/49 (8·2)
	(N.S.)	
14	21/43 (48·8)	9/48 (18·8)
	$(P<0{\cdot}01)$	
21	22/39 (56·4)	12/47 (25·5)
	$(P<0{\cdot}01)$	
28	22/37 (59·5)	12/42 (28·6)
	$(P<0{\cdot}02)$	

* Percentage in parentheses. N.S. = not significant.

TABLE 4. Percentage of patients showing complete disappearance of different signs and symptoms at the end of the study period (28th day of follow up)

	% of patients showing disappearance of signs/symptoms	
Signs/symptoms	Ciclopirox	Placebo
Redness	27/33 (81·8)*	18/27 (66·7)
	(N.S.)	
Burning	5/5 (100)	0/1 (0)
	(N.S.)	
Itching	21/34 (61·8)	12/40 (30·0)
	($P<0\cdot05$)	
Vesicles	23/25 (92·0)	14/27 (51·9)
	($P<0\cdot01$)	
Scaling	19/36 (52·8)	11/41 (26·8)
	($P<0\cdot05$)	

* Percentage in parentheses. N.S. = not significant.

7, 14, 21 and 28 as compared to the placebo group. Similarly, a greater number of patients in the Ciclopirox group showed negative culture as compared to those in the placebo group, the P value being 0·05 on days 14 and 21.

The results have also been analysed correlating the clinical assessment given by the investigator with negative microscopic examination. For this, only those patients are considered cured who scored either marked improvement or complete disappearance of signs and symptoms together with a negative microscopic examination in different days of follow-up (Table 3). It will be seen that Ciclopirox proved to be significantly better than placebo throughout the period of observation. So far as the individual signs and symptoms are concerned, significant amelioration of itching, vesicles and scaling was achieved with Ciclopirox as compared to the placebo (Table 4).

None of the patients in either the Ciclopirox- or the placebo-treated group showed any local or systemic side-effects. Haematological examination and liver and kidney function tests carried out before and after the treatment did not reveal any abnormality.

DISCUSSION

Our results clearly demonstrate the efficacy of Ciclopirox. The compound is shown to be effective against infections with *Trichophyton rubrum*, *T. violaceum*, *T. mentagrophytes*. In cases of pityriasis versicolor caused by *Malassezia furfur*, although Ciclopirox produced a favourable response, further confirmation is required in view of the small number of patients. Ciclopirox has produced a superior effect as compared to placebo according to all different criteria used to assess the efficacy.

These results agree with a number of uncontrolled clinical reports in which Ciclopirox was studied in patients with superficial mycoses. A summary of these reports is given in Table 5, which shows a cure rate between 80·9 and 93·75%.

Satisfactory regression of symptoms, such as redness, burning, itching, vesicles and scaling, was

TABLE 5. Summary of some of the clinical studies on Ciclopirox 1% solution

Investigator	Clinical diagnosis	No. of patients	Duration of application (days)	% of patients showing cure
Defina & Pereira (1974)	Dermatophytoses	36		
	Candidiasis	4		
	Pityriasis versicolor	2	19·5	100
	Total	42		
Ermitice *et al.* (1974)	Dermatophytoses	21	21	90·5
Pereira & Tarle (1974)	*P. versicolor*	9	21–28	80·9
	Dermatophytoses	9		
	Candidiasis	3		
	Total	21		
Porto & Braga (1974)	Dermatophytoses	25	21	88
Moraes (1974)	*T. rubrum*	17	21	93·75
	M. furfur	6		
	T. mentagrophytes	3		
	E. floccosum	2		
	C. albicans	2		
	M. canis	1		
	Total	32		
Minelli, Piraino & Schnitzler (1974)	*T. pedis*	34	21	79·4
	T. cruris	18		
	P. versicolor	12		
	T. corporis	11		
	C. albicans	8		
	Miscellaneous	14		
	Total	97		

observed in the group receiving Ciclopirox. The symptoms which responded most significantly to Ciclopirox were itching, vesicles and scaling.

Ciclopirox when applied topically was found to be devoid of such side-effects as burning, itching and redness. No systemic side-effects were observed as evaluated by liver and kidney function tests and haematology.

Ciclopirox (HOE 296) can be considered an effective and safe anti-mycotic agent for the treatment of dermatomycoses.

ACKNOWLEDGMENTS

The author expresses his gratitude to Dr Inderjeet Singh, M.D. Assistant Professor, Department of Pathology and Microbiology, for laboratory help. Thanks are also due to Dean, Goa Medical College, Goa, for his permission to publish this communication.

REFERENCES

DEFINA, A.F. & PEREIRA, C.C.A. (1974) Emprego de novo antimicotico piridonico, o HOE-296, no tratamento topico de micoses superficiais. *Revista Brasileira de Clinica E Terapeutica*, 3, Suppl. 7, 7.

ERMETICE, L.N., NANNI, M.E., CARBONARI, M. & CASTRO, R.M. (1974) Avaliacao terapeutica de um novo antimicotico piridonico de use topico, HOE-296, em dermatofitoses. *Revista Brasileira de Clinica E Terapeutica*, 3, Suppl. 7, 15.

MINELLI, L., PIRAINO, R. & SCHNITZLER, R. (1974) Tratamento topico das micoses superficials pelo HOE-296 um novo antimicotico piridonico. *Revista Brasileria de Clinica E Terapeutica*, 3, Suppl. 7, 23.

MORAES, M.L. (1974) Resultados clinico e micologicos obtidos com os uso do ciclopirox (HOE-296) em micoses superficiais. *Revista Brasileira de Clinica T Terapeutica*, 3, Suppl. 7, 27.

PEREIRA, L.C. & TARLE, S.F. (1974) Ensaio clinico a terapeutico com um novo antimicotico piridonico de empergo topico, o HOE-296. *Revista Brasileira de Clinica E Terapeutica*, 3, Suppl. 7, 3.

PORTO, J. & BRAGA, M.P. (1974) Consideracoes terapeuticas sobre o uso de um novo antimicotico piridonico, o HOE-296, no tratamento topico de dermatofitoses. *Revista Brasileira de Clinica E Terapeutica*, 3, Suppl. 7, 19.

ROMITI, N. (1974) HOE-296, um novo antimicotico piridonico de emprego topico, com atividade anti-bacteriana adicional. *Revista Brasileria de Clinica E Terapeutica*, 3, Suppl. 7, 1.

British Journal of Dermatology (1976) **95**, 89.

Case Reports

Prurigo nodularis and gluten enteropathy

A.W.McKENZIE, D.G.STUBBING AND B.L.ELVY

Norfolk and Norwich Hospital, Norwich

Accepted for publication 6 October 1975

SUMMARY

Two cases of prurigo nodularis are described. Each is considered to have a gluten sensitive enteropathy. This association may be one facet of prurigo nodularis.

'A Case of Multiple Tumours of the Skin Accompanied by Intense Itching' was published by Hardaway in 1880, and the condition given the name Prurigo Nodularis by Hyde in 1909. Brocq probably described the same entity in 1900 as Lichen Obtusus Corneus. Since then factors mainly related to the psyche have been described as of aetiological significance: 'Le facteur constitutionnel émotif est certain...' (Duperrat, 1959); 'The disease is regarded as an atypical form of neurodermatitis circumscripta ... Most patients are physically depleted and nervously exhausted...' (Domonkos, 1971); '...the condition has rightly been referred to as neurotic excoriation' (Borrie, 1967). Fitzpatrick *et al.* (1971) cite 'repeated local trauma' as important, while Rook, Wilkinson & Ebling (1972), in mentioning emotional stress, point out that there is no definite evidence of this being more than a contributory factor.

We have recently studied two patients who fit the usual clinical criteria of prurigo nodularis. We feel that each has a gluten enteropathy and suggest, on the basis of these two individuals and other sparse reports in the literature, that the association may not be fortuitous and that prurigo nodularis and gluten enteropathy may be one facet of the prurigo group.

Case 1. *Mrs V.C. Aged* 41 *years.* In 1955, when aged 22 years, the patient developed an itchy papular eruption on the extensor aspect of her limbs. Worsening appeared to occur in the winter and a diagnosis of prurigo hiemalis was made. Over the next 4 years the prurigo became continuous and from 1959 to 1973, with frequent reference being made to her agitated mental state, the eruption persisted. Relief was obtained for short periods only by five hospital admissions and the use of topical steroids and polythene occlusion. Therapeutic trial with dapsone on two occasions gave no easing of her pruritus. In June 1973 macrocytosis was found and she was admitted to hospital for investigation. Examination revealed a woman of slender build, height 1 m 61 cm, and weight 46 kg. She was adamant that pin-point spots appeared initially and that she then scratched. She was unable to decide if her bouts of intense irritation were the cause or result of her periodic anxiety symptoms. Keratotic and excoriated prurigo nodules, 1–2 cm in size, were widely dispersed over the extensor aspect of her arms and legs and over the back of her lower chest. Neither the nodules nor the remainder of her skin was hyper-pigmented. She had one bowel movement each day of normal consistency. She had one female sibling who had hay fever. There was no family history of bowel disorder. A 4-year-old daughter was well.

Investigations. Relevant abnormal findings were: Haemoglobin 11·3 g%; M.C.V. 101 μ^3; E.S.R. 12 mm in 1st hour; blood film showed macrocytes and polychromasia; R.B.C. folate 90 ng/ml (normal greater than 160 ng/ml); serum iron 38 μg/100 ml (N.R. 65–175); serum iron binding capacity 345 μg/100 ml (N.R. 250–420); serum carotene 50 μg/100 ml (N.R. 50–200); serum vitamin B12 158 Pg/ml (N.R. 170–800); oral glucose tolerance showed negligible rise of 10 mg/100 ml; faecal fat (3 day collection) 12·6 g/24 h (N.R. up to 5·0 g/24 h); no IgA deposits on direct immunofluorescence; skin biopsy showed hyperkeratosis, acanthosis and prolongation of the rete ridges with chronic inflammatory changes in the dermis, findings consistent with prurigo nodularis; jejunal biopsy showed a thin mucosa, sparse, short and rounded villi with a slight increase in chronic inflammatory cells in the lamina propria. The appearance was that of a gluten enteropathy.

A gluten-free diet was started in September 1973 and she reported an increased sense of well-being after 3 months, together with progressive increase in weight. In January 1975 a faecal fat level was normal (2·2 g/24 h) and repeat jejunal biopsy showed less stunting than previously but not the attainment of normal length. Serum iron and red cell folate level have returned to normal. Almost 2 years after the institution of a gluten-free diet she still has prurigo but is much less troubled by it.

Case 2. Mrs R.B. Age 37 *years*, was admitted to the Norfolk and Norwich Hospital on 11.1.75 as an emergency in a 'pruritic crisis'. At the age of 29 years she had developed an itchy rash on both elbows with subsequent spread to involve the limbs extensively and the lower trunk to a lesser extent. She had been treated intermittently at another hospital for 'nummular eczema', with dermatitis herpetiformis as a possible alternative diagnosis. She was adamant that when lesions appeared they never resolved completely, and described bouts of exquisite itching of the skin from time to time. Over the last 5 years she had been anaemic and had had unsuccessful treatment with oral iron. Her bowel function was normal and uncomplicated pregnancies occurred in 1963 and 1964. She had four siblings: a brother was in good health, but each of her three sisters had a bowel disorder. One, aged 40 years, had Crohn's disease, one, aged 38 years, had been shown to have the atrophic mucosa of coeliac disease in adult life and took a gluten-free diet, while the other, aged 31 years, had coeliac disease diagnosed when aged 3 years in 1948 at University College Hospital and took a gluten-free diet. None of the siblings had any skin complaints, nor was there any bowel disorder in the siblings' eleven offspring.

Examination revealed 1–2 cm prurigo nodules affecting the extensor and lateral aspects of the limbs and the lower back and buttocks of a patient 1 m 60 cm in height and weighing 56·95 kg.

Investigations. Relevant abnormal findings were: Haemoglobin 10·0 g% with a film showing a mixed picture of microcytosis, hypochromia and slight macrocytosis; E.S.R. 23 mm in 1st hour; serum iron 23 μg/100 ml (N.R. 65–175); iron binding capacity 400 μg/100 ml (N.R. 250–420); serum folate 1·3 μg/ml (N.R. greater than 3 μg/ml); R.B.C. folate 120 ng/ml (N.R. >160 ng/ml); xylose absorption test—urinary xylose 1·2 g/5 h (N.R. 4–8); serum carotene 40 mg/100 ml (N.R. 50–200); faecal fat (3 day collection) 12·2 g/24 h (N.R. up to 5 g/24 h); serum IgG 1,925 mg/100 ml (N.R. 800–1800); no IgA deposits on direct immunofluorescence; jejunal biopsy—'Severe villous atrophy as seen in idiopathic steatorrhoea'; skin biopsy—'Hyperkeratosis and moderate acanthosis with elongation of rete ridges. Low grade chronic inflammation of the underlying dermis. The appearances are consistent with prurigo nodularis.' Barium meal and follow through showed mucosal pattern and irregular dilatation in the jejunum and ileum suggestive of steatorrhoea.

A therapeutic trial with dapsone produced no relief of symptoms. A gluten-free diet was instituted on 22.1.75 together with folic acid 5 mg three times daily. The patient reported an immediate increased sense of well-being, together with weight gain. A repeat jejunal biopsy on 22.7.75 showed no improvement in her atrophic mucosa but a 3-day stool collection showed that fat excretion had returned to normal (1·9 g/24 h). She continues to have prurigo but is very much less troubled by it.

DISCUSSION

Prurigo nodularis is a relatively rare but distinctive disorder in which hard, keratotic, globular nodules, 1–3 cm in diameter, are found in symmetrical distribution on the extensor aspect of the limbs, although the trunk may also be involved. The majority of instances are described in middle-aged women. The intervening skin is mostly normal in appearance and there is no generalized pigmentation. The nodules occasion bouts of intense pruritus leading to ferocious scratching which, in association with the histology, has made some equate the entity with 'localized lichen simplex chronicus' (Shaffer & Beerman, 1951). Histologically the main features are those of lichen simplex with hyperkeratosis, acanthosis and sometimes pseudo-epitheliomatous hyperplasia. Changes in the morphology of terminal nerves were described by Cowan (1964) in prurigo nodularis, but he found similar hyperplasia of nerve fibres and Schwann cells in lichen simplex chronicus. The changes are probably the result of scratching. Reference has been made earlier to aetiological factors suggested by some standard tests.

In his review of skin disease occurring with steatorrhoea, Wells (1962) gave detailed histories of six of his own cases. In five instances the skin manifestations were eczematous and mainly discoid and intertriginous in distribution, and of these four showed marked pigmentation. The eczema cleared with treatment of the anaemia and steatorrhoea. In the sixth case he described a female of 44 years who had prurigo nodularis of 7 years' duration associated with intermittent atopic eczema. Her skin cleared when she was treated with a low fat diet and folic acid supplements. In 1965 a jejunal biopsy was performed showing a flattened mucosa consistent with coeliac disease, and she subsequently had a gluten-free diet and folic acid supplements with no recurrence of her prurigo (Wells, 1975).

Howell (1967) described a female aged 53 years who, after many years of 'colitis', was shown to have a gluten enteropathy associated with a 3 year history of 'exudative nummular prurigo' combined with hyperpigmentation of the neck and elbows. Appropriate treatment of her gut plus local skin therapy lead to her having 'only very mild residual skin lesions'.

Apart from these two cases and the two patients who form the basis of this report, we can find no other instance of a similar combination of skin and gut signs. We feel that there is sufficient proof of our first patient having coeliac disease due to gluten sensitivity. She has gained weight, her biochemical evidence of malabsorption has returned to normal, and there has been improvement in her jejunal mucosa biopsy, although not complete return to normality. The return of the atrophic mucosa of coeliac disease to histological normality with a gluten-free diet is not well documented, but periods ranging from months to 2 years are said to be necessary (Creamer, 1974). Sometimes the villous structure remains abnormal. By the same criteria we feel that our second patient, having two sisters with coeliac disease and a normal fat excretion after gluten exclusion, has a gluten enteropathy. The familial incidence of coeliac disease was documented by Mylotte *et al.* (1974) who found histological changes of coeliac disease in 11% of first degree relatives. By the usually accepted criteria, which are clinical rather than histological, both patients have the rare syndrome of prurigo nodularis. The absence of IgA deposits in uninvolved skin, taken with other factors, rules out dermatitis herpetiformis as a diagnosis (Fry & Seah, 1974).

It is not possible to be dogmatic about a possible genetic link between prurigo nodularis and coeliac disease or, on the basis of our two cases, to present it as a parallel with dermatitis herpetiformis and its gluten enteropathy. We are convinced that prurigo nodularis and coeliac disease exist in our patients, but acknowledge that further studies are required to confirm a relationship. Improvement, but not complete clearing of skin lesions, has occurred so far on a gluten-free diet.

REFERENCES

BORRIE, P. (1967) *Roxburgh's Common Skin Diseases*, 13th edn, p. 367, H.K.Lewis & Co., London.

BROCQ, L.A.J. (1900) *Pratique Dermatologique*, 3, 201.

COWAN, M.A. (1964) Neurohistological changes in prurigo nodularis. *Archives of Dermatology*, **89**, 954.

CREAMER, B. (1974) The small intestine. *Tutorials in Postgraduate Medicine*, Vol. 4, p. 104. Heinemann, London.

DOMONKOS, A.N. (1971) *Andrews' Diseases of the Skin*. 6th edn, p. 69. W.B.Saunders, Philadelphia.

DUPERRAT, B. (1959) *Précis de Dermatologie*, 6th edn. Masson et Cie, Paris.

FITZPATRICK, T.B., ARNDT, K.A., CLARK, W.H., EISEN, A.Z., VAN SCOTT, E.J. & VAUGHAN, J.H. (1971) *Dermatology in General Medicine*, p. 666. McGraw-Hill, New York.

FRY, L. & SEAH, P.P. (1974) Dermatitis herpetiformis: an evaluation of diagnostic criterion. *British Journal of Dermatology*, **90**, 137.

HARDAWAY, W.A. (1880) A case of multiple tumours of the skin accompanied by intense itching. *Archives of Dermatology*, **6**, 129.

HOWELL, R. (1967) Exudative nummular prurigo with idiopathic malabsorption syndrome. *British Journal of Dermatology*, **79**, 357.

HYDE, W. (1909). *Diseases of the Skin*, 8th edn. Lea & Febiger, New York.

MYLOTTE, M., EGAN-MITCHELL, B., FOTTRELL, P.F., MCNICHOLL, B. & MCCARTHY, C.F. (1974) Family studies in coeliac disease. *Quarterly Journal of Medicine*, **43**, 359.

ROOK, A., WILKINSON, D.S. & EBLING, F.J.G. (1972) *Textbook of Dermatology*, 2nd edn, Vol. 1, p. 292. Blackwell Scientific Publications, Oxford.

SHAFFER, B. & BEERMAN, H. (1951) Lichen simplex chronicus and its variants. *Archives of Dermatology and Syphilis*, **64**, 340.

WELLS, G.C. (1962) Skin disorders in relation to malabsorption. *British Medical Journal*, **iv**, 937.

WELLS, G.C. (1975) Personal communication.

British Journal of Dermatology (1976) **95**, 93.

Sarcoidosis in a child, presenting as an erythroderma with keratotic spines and palmar pits

J.G.L.MORRISON*

Department of Dermatology, University of Pretoria, Pretoria

Accepted for publication 30 October 1975

SUMMARY

A case of sarcoidosis with generalized erythroderma, exfoliation and micropapule formation in a 6-year-old boy is presented. Follicular spiny keratoses, resembling those seen in pityriasis rubra pilaris, and palmo-plantar pitting were among the other extraordinary features in this case. The epidermis overlying the widespread sarcoid granulomata showed parakeratosis, lymphocytic infiltration and degeneration of the basal layer.

Sarcoidosis with generalized exfoliation and universal erythroderma is most unusual. The so-called erythrodermic forms of sarcoidosis are generally patchy, and scarcely merit the description of an erythroderma (Bernhardt & Zalewski, 1934; Rischin, 1922). Large erythrodermic sheets may occur, however (Schaumann, 1924; Wigley & Musso, 1951; Lever & Freiman, 1948). A patient with erythrodermic sheets with generalized exfoliation was described by Simpson (1963) who suggested that the exfoliation was an allergic reaction to a toxin. According to Degos (1972) and Degos *et al.* (1953) universal erythroderma as a manifestation of sarcoidosis has been recorded three times, although the references given for these publications were not explicit. Tracing them to their sources as well as we were able, it transpired that none of them were cases of generalized erythroderma and exfoliation. Of these, Watrin & Masson's (1948) patient bears the closest resemblance to our case, though exfoliation was not a feature.

CASE REPORT

The patient, a 6-year-old Portuguese boy, had suffered from universal erythroderma and exfoliation for 18 months. Exfoliation and oedema impaired his facial movements (Fig. 1). His scalp was covered by large scales which matted his hair. In addition to the generalized scaling, his skin was covered with multiple small papules which were best seen after treatment had lessened the scaling (Fig. 2). On the dorsa of his hands and fingers and on the posterior part of his neck there were keratotic spines resembling those seen in pityriasis rubra pilaris. Random skin biopsies were taken from numerous areas, and sarcoid granulomata were identified in all these specimens. The granulomata were mostly situated in the papillary dermis, and affected the overlying epidermis. They consisted of epithelioid

---

* Present address: St John's Hospital for Diseases of the Skin, Lisle Street, London WC2H 7BJ.

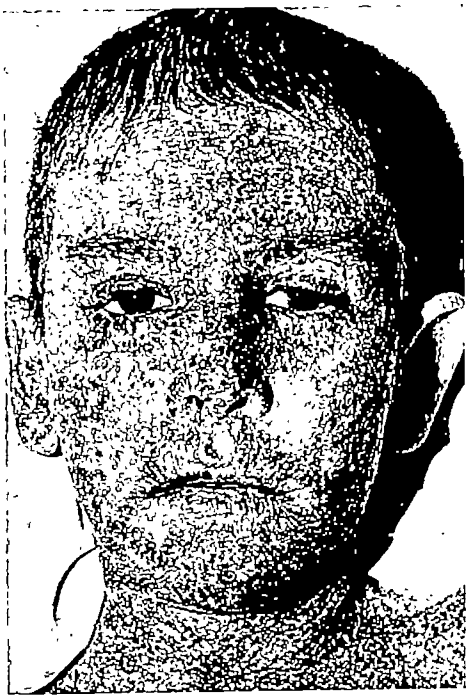


FIGURE 1. Sarcoidosis: exfoliation and oedema impairing facial movements.

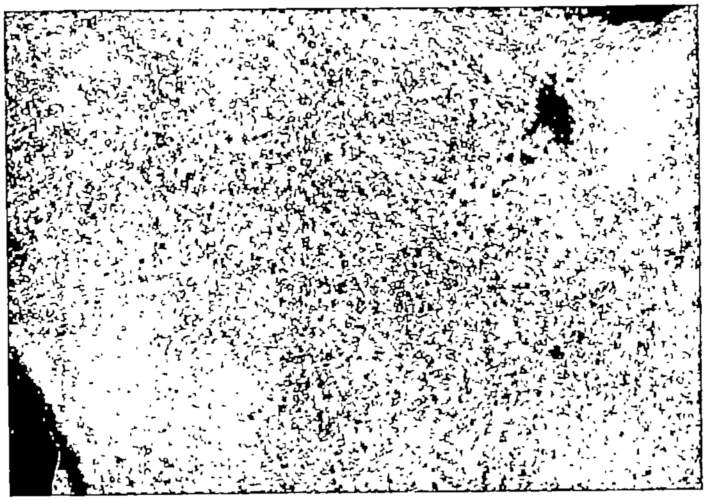


FIGURE 2. Sarcoidosis: innumerable micropapules on the upper chest.

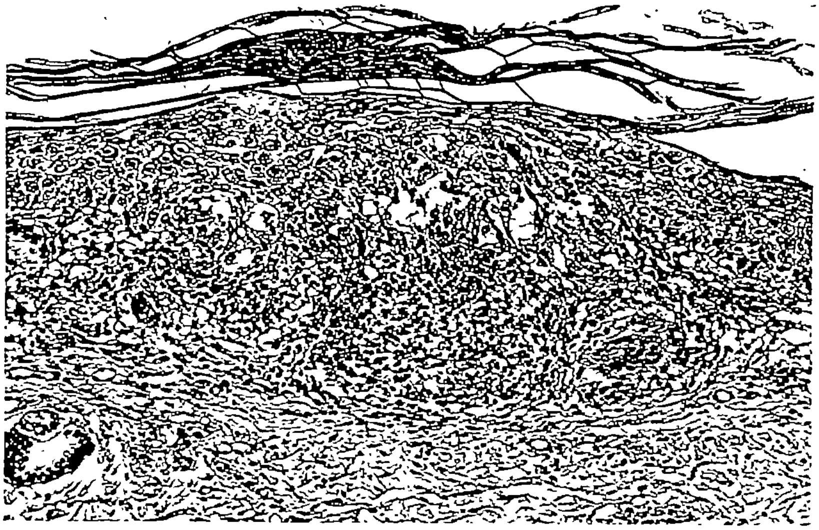


FIGURE 3. A sarcoid granuloma involving the overlying epidermis with degeneration of the lower layers, lymphocytic infiltration, spongiosis and parakeratosis (× 180).

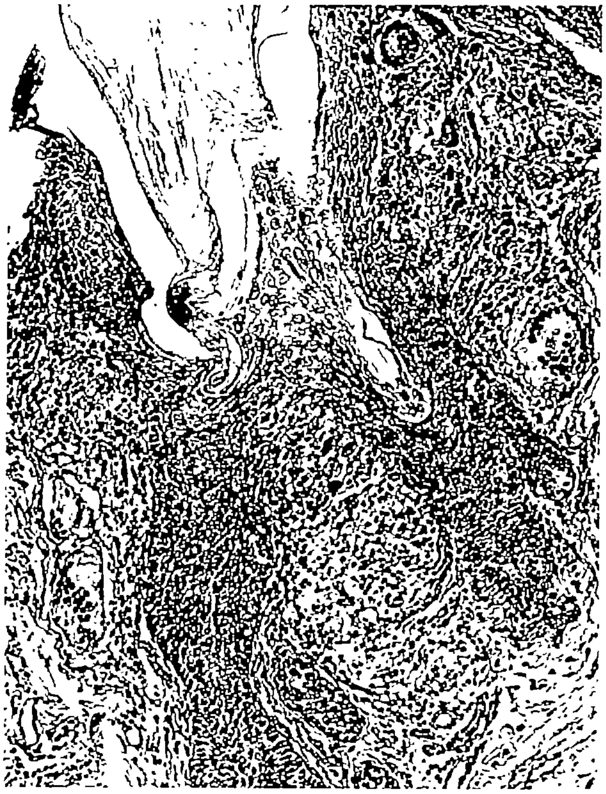


FIGURE 4. Hair follicle plugged with keratin and associated with a sarcoid granuloma (× 180).

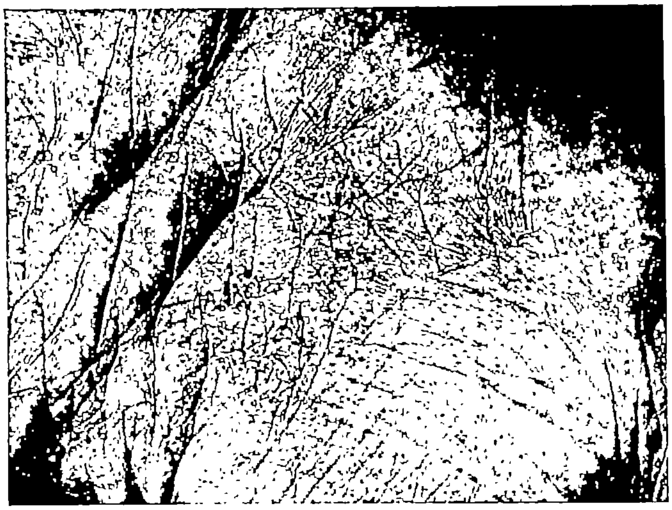


FIGURE 5. Sarcoidosis: numerous palmar pits.

cells, lymphocytes and Langhans' giant cells, many containing Schaumann inclusion bodies. The epidermis showed parakeratosis, spongiosis, lymphocytic infiltration and degeneration of the basal and lower spinous layers (Fig. 3). Hair follicles associated with these granulomata were plugged with keratin, giving rise to the keratotic spines (Fig. 4). The patient did not show palmo-plantar hyperkeratosis as might have been expected in pityriasis rubra pilaris, but instead showed palmo-plantar pits as seen in the basal cell naevus syndrome (Fig. 5). Histology of these palmar pits showed an area of parakeratosis with invagination of the epidermis, which was not atrophic, into the dermis. The epidermis thus formed a cup filled with the parakeratotic keratin. Pit formation occurs when the parakeratotic keratin loosens and is lost. The pathogenesis of the parakeratotic growth is, however, unclear. Sarcoid granulomas were present in the mid-dermal region but none in the region of the dermo-epidermal junction where one would have expected them to be. Only a mild, unorganized inflammatory infiltrate was present.

Two years before, the patient had suffered from painful swollen joints. The wrists and knees were most severely affected. A synovial biopsy from his wrist had shown a subacute synovitis but no synovial proliferation or granulomata. The joints were normal on X-ray.

A generalized lymphadenopathy and splenomegaly were present. The glands were smooth, firm and freely mobile. His splenomegaly was difficult to interpret, as he had apparently also suffered from malaria.

Sore eyes and headache were also troublesome. Present were bilateral punctate corneal opacities, unilateral iris nodules and posterior synechiae, the probable result of a previous uveitis.

A chest X-ray revealed a diffuse, fine symmetrical fibrosis in the lung bases. He was anergic to the Tine (tuberculin) test.

Laboratory tests

Rheumatoid factor and anti-nuclear antibodies were negative while the serum calcium, phosphate, protein and immunoelectrophoresis were within the normal limits.

Treatment

The skin lesions readily improved on local and systemic corticosteroid treatment. Prednisolone 4 mg daily was given for 10 months. The condition improved to such an extent that he failed twice to report for follow up, believing himself to have been cured. On both occasions he suffered a relapse within 4 weeks without treatment before reporting to the clinic.

Although the erythroderma and scaling disappeared on the corticosteroid treatment, papulation was still visible in places.

DISCUSSION

Sarcoidosis in children under the age of 9 years is rare (Kendig & Wiley, 1961). Organs that are frequently involved in children are skin (27%), lymph glands (70%), hilar lymph nodes and lung (100)%, liver (33%) and eyes (24%). Joint swelling was also noted on one occasion (Kendig, 1974).

In addition to the erythroderma this patient had evidence of sarcoidosis in a number of organs other than the skin. Involved were the eyes, joints, lungs, lymph glands and possibly spleen. Histologically sarcoid granulomata could be demonstrated in all six random skin biopsies. Except for the palms and soles the granulomata were situated at the dermo-epidermal junction. In this position they obviously could give rise to the epidermal changes. The patient took no medication before the onset or during the course of his illness which could be suspected of causing his erythroderma. We therefore regard sarcoidosis as the cause of his erythroderma.

Keratotic spines on the backs of the thighs and about the buttocks associated with sarcoidosis have been reported in an adult (Wigley & Musso, 1951). The spine-like keratoses that appeared on our patient's hand dorsa and posterior part of the neck, led to the initial diagnosis of pityriasis rubra pilaris in the erythrodermic phase. Palmo-plantar pits were a prominent feature in our patient and appeared to be a novel finding.

REFERENCES

BERNHARDT, R. & ZALEWSKI, J. (1934) Zür Kenntnis der erythrodermischen Form der Boeckschen Krankheit. *Archiv für Dermatologie und Syphilis*, 170, 226.

DEGOS, R., LORTAT-JACOB, E., DELZANT, O., HEWITT, J. & LABET, R. (1953) Maladie de Schaumann: forme érythroparakeratosique (érythème sarcoïdique). Association à un prurigo nodularie (prurigo de la maladie de Schaumann ?). *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, 60, 410.

DEGOS, R. (1972) *Dermatologie*, p. 533. Flammarion, Paris.

KENDIG, E.L. & WILEY, E.J. (1961) Sarcoidosis in children. *Postgraduate Medical Journal*, 37, 590.

KENDIG, E.L. (1974) The clinical picture of sarcoidosis in children. *Pediatrics*, 54, 289.

LEVER, W.F. & FREIMAN, D.G. (1948) Sarcoidosis. *Archives of Dermatology and Syphilogy*, 57, 639.

RISCHIN, M. (1922) Universelles benignes Miliarlupoid Boeck mit Beteiligung innerer Organe. *Archiv für Dermatologie und Syphilis*, 139, 30.

SCHAUMANN, J. (1924) Benign lymphogranuloma and its cutaneous manifestations. *British Journal of Dermatology*, 36, 521.

SIMPSON, J.R. (1963) Sarcoidosis with erythrodermia and ulceration. *British Journal of Dermatology*, 75, 193.

WATRIN, J. & MASSON, R. (1948) Maladie de Besnier-Boeck-Schaumann à localisations cutanées généralisées à tout le corps. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, 55, 343.

WIGLEY, J.E.M. & MUSSO, L.A. (1951) A case of sarcoidosis with erythrodermic lesions. Treatment with calciferol. *British Journal of Dermatology*, 63, 398.

British Journal of Dermatology (1976) **95**, 99.

Brief Communications

Pemphigus-like syndrome induced by D-penicillamine

S.G.TAN AND N.R.ROWELL *Department of Dermatology, General Infirmary, Leeds*

D-Penicillamine is effective in the treatment of Wilson's disease, cystinuria and lead poisoning, and it is promising in that of rheumatoid arthritis. In the last decade many unwanted effects of D-penicillamine have been recorded—loss of taste, nephrotic syndrome, lupus erythematosus-like syndrome and agranulocytosis. Since 1969, bullous eruptions occurring in ten patients who received D-penicillamine have been reported from France (Hewitt, Benveniste & Lessana-Leibowitch, 1975). However, there is some doubt as to whether all the cases described were pemphigus-like syndrome and not toxic epidermal necrolysis (Lyle, 1975). We, therefore, thought it worthwhile reporting a patient who suffered from pemphigus-like syndrome during treatment with D-penicillamine.

Case report

A 59-year-old lady, who had had seropositive rheumatoid arthritis for over 2 years, was started on D-penicillamine (Dista) 1250 mg, copper sulphate 15 mg, and soluble aspirin 900 mg daily in February, 1975. Two months later she noticed blisters and sore areas on her trunk and arms. When the dose of D-penicillamine was gradually reduced, there were fewer blisters. In July, 1975, examination showed occasional bullae measuring 1 × 1 cm, multiple raw areas and crusts on the trunk and upper arms; the surface of the bullae was easily dislodged with sliding pressure. No buccal lesions were seen. Histological examination showed intra-epidermal bullae and large areas of parakeratosis above the well-formed granular layer. Immunohistological sections revealed intra-epidermal bullae with intercellular staining for IgG and C3.

As long as D-penicillamine was given (250 mg daily) occasional bullae continued to appear. When it was stopped, in September 1975, the bullae virtually resolved within a few weeks. The skin was completely clear in October, 1975, and her arthritis was controlled with aspirin alone.

Discussion

Although D-penicillamine is of help in the treatment of Wilson's disease, cystinuria, lead poisoning and some patients with rheumatoid arthritis, many unwanted effects such as nephrotic syndrome and myasthenic reactions have been reported. To date, bullous eruptions have been recorded in Paris in ten patients who received D-penicillamine: nine of the patients had had rheumatoid arthritis and one Wilson's disease. The present patient with pemphigus-like syndrome induced by D-penicillamine is the first to be reported in the United Kingdom.

There is no doubt that this patient suffered from pemphigus-like syndrome as shown by the clinical, histological and immunohistological features. Pemphigus antibody was not detected in the serum, although of the six patients reported from Paris as showing pemphigus antibody, five had a low titre and one had a titre of 1/1600. It would appear that the lesions were dose-dependent. The number of lesions decreased, but lesions continued to appear when the dose was reduced from 1250 mg to 250 mg daily. The condition resolved completely on stopping the drug.

Since the skin lesions in this patient resolved with the complete withdrawal of D-penicillamine and the arthritis was controlled with soluble aspirin, drugs such as prednisolone or azathioprine were not given. The bullous eruptions in some of the patients reported from Paris responded to moderate doses of corticosteroids or cyclophosphamide, whilst in others it was difficult to control.

Pemphigus-like syndrome is yet another unwanted effect of D-penicillamine, but the drug should not be withheld from patients who may benefit from it; for example, patients with Wilson's disease or resistant rheumatoid arthritis. Thus, D-penicillamine should only be prescribed by clinicians who are familiar with its hazards (Leading article, *Lancet*, 1975). It should be started in a dose of 250 mg daily with small increments at long intervals (Leading article, *British Medical Journal*, 1975).

REFERENCES

HEWITT, J., BENVENISTE, M. & LESSANA-LEIBOWITCH, M. (1975) Pemphigus induced by D-penicillamine. *British Medical Journal*, iii, 371.

Leading article (1975) Penicillamine. More lessons from experience. *British Medical Journal*, iii, 120.

Leading article (1975) D-penicillamine in rheumatoid arthritis. *Lancet*, i, 1123.

LYLE, W.H. (1975) Personal communication.

Beta-carotene in the treatment of discoid lupus erythematosus

PETER C.H.NEWBOLD *Department of Dermatology, Worcester Royal Infirmary, Castle Street, Worcester WR1 3AS*

Beta-carotene has been used in the treatment of photodermatoses since its success in erythropoietic protoporphyria (EPP) (Mathews-Roth *et al.*, 1970). In addition to EPP, I have found the drug of considerable value in cases of solar urticaria, and of benefit in some patients whose eczema flares after light exposure. A recent review summarizes the clinical data available, as well as the known biochemistry and pharmacology (Pollitt, 1975).

The summer of 1975 was exceptionally clear and sunny, and produced a large harvest of patients with sun-provoked symptoms. Amongst these were several with discoid lupus erythematosus whose control became unsatisfactory. I tried beta-carotene in three such patients, and report the results briefly because they were so successful.

Patient 1 (Mrs S.E., aged 53 years)

She had a 12-year history of chronic discoid lupus erythematosus, which initially responded to chloroquin. This had to be stopped because of ocular damage. Subsequently she was not controlled by mepacrine 50 mg/day, together with local steroids and light-protective creams. She came 'to dread the light', and in May her skin began to flare, so that by early July the whole of her face looked as if it had been boiled. The forehead, nose, cheeks, and chin showed confluent plaques of active lupus erythematosus, with much oedema and erythema. Beta-carotene 50 mg three times daily was started, and there was a response (noted first by her husband) in 1 week. After 1 month of treatment the skin looked normal, she was free from discomfort, the skin was supple and soft, and the erythema had faded. She was even able to venture out of doors in the autumn sunshine, and her life has been transformed—to use her own words. There were no side-effects.

Patient 2 (Mrs A.C., aged 45 years)

This lady had had discoid lupus erythematosus for 23 years, initially only on the face and neck, and giving little trouble until 3 years ago. It then became more active, new lesions appeared, especially on her scalp, and the older ones enlarged. Topical steroids, combined with the use of Uvistat before light exposure, did not help her at all. She was started on chloroquin sulphate 200 mg twice daily, with initial relief. This had to be stopped after 5 weeks because of nausea, retching, photophobia, and weakness in the legs. After the chloroquine was stopped, she flared severely over the face and neck, and when seen in July she was both unsightly and depressed. She was given beta-carotene 50 mg three times daily, and had complete comfort within 2 weeks. This was maintained and all the lesions have regressed satisfactorily. She has been able to discontinue local steroids, and has had no side-effects from the beta-carotene, which was stopped after 2 months.

Patient 3 (*Mrs F.S., aged* 58 *years*)
This patient with an 18-year history of discoid lupus erythematosus, mainly on the cheeks, had been well controlled with Synalar Forte and Uvistat. However in August there was an inflamed extension of her chronic lesions, and many new ones over her scalp, chin and neck. She was unable to lead a normal life, was in constant discomfort, and had to cancel her holiday. Because of the success of the first two patients, she was started on beta-carotene 50 mg three times daily, together with 1% hydrocortisone lotion for the painful areas. After 1 week she was much more comfortable; many lesions had cleared after 2 weeks, and after 3 weeks the initial diagnosis would have been doubted. She stopped treatment after 2 months, and had had no side-effects.

Comment
I know of no other reports of the use of beta-carotene in lupus erythematosus, a common disease which can be hard to manage. These results suggest that a wider trial is justified, especially in those patients unable to use chloroquin. The speed of response and the lack of side-effects are especially attractive features.

Acknowledgment
I am grateful to Dr Norman Pollitt of Roche Products Ltd., Welwyn Garden City, for supplies of beta-carotene capsules.

REFERENCES

MATHEWS-ROTH, M.M., PATHAK, M.A., FITZPATRICK, T.B., HARBER, L.C. & KASS, E.H. (1970) β-carotene as a photoprotective agent in erythropoietic protoporphyria. *New England Journal of Medicine*, **282**, 1231.
POLLITT, N. (1975) β-carotene and the photodermatoses. *British Journal of Dermatology*, **93**, 721.

Systemic use of a new retinoid with and without local dithranol treatment in generalized psoriasis

C.E.ORFANOS AND U.RUNNE *Department of Dermatology, University of Cologne, Cologne, West Germany*

Over the past few years numerous clinical and laboratory investigations have been undertaken in this hospital to evaluate the systemic and topical application of retinoic acid and its derivatives (retinoids) in psoriasis. Some results have been published previously, indicating that retinoic acid alone does not clear psoriatic lesions, although some improvement may be noted. Moreover, frequent irritation reduced its clinical efficacy (Runne, Orfanos & Gartmann, 1973; Orfanos *et al.*, 1973).

Recently, a new aromatic retinoid [ethyl-all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3-7-dimethyl-2,4,6,8-nona-tetraenoate, RO 10/9359, Hoffman-La Roche, Basle] has been synthesized, which seemed promising after systemic application in experimental work with animals (Bollag, 1974a,b). Preliminary clinical work confirmed its ability to reduce epithelial proliferation and showed that the new compound had beneficial effects in generalized psoriasis (Ott & Bollag, 1975). The side-effects of the drug were moderate in these studies, in doses up to 100 mg/day. In particular headaches, arthralgia and elevation of liver enzymes did not occur after prolonged medication.

We decided, therefore, to employ the new retinoid systemically in twelve adults with widespread psoriasis, who were hospitalized for clinical treatment. Most of them had been treated earlier with anthralin or topical corticosteroids. They were all men, 21–81 years in age. Local treatment was in six cases 2% salicylic acid in petrolatum; the other six patients were treated simultaneously with low

TABLE 1. Patients treated with 75–100 mg retinoid orally per day

Patient	Age	Type of psoriasis	Other diseases	Duration of oral treatment (weeks)	Local* treatment	Result†	Observations during therapy
1	67	Generalized	No	4	S.A.	Insufficient	Cheilitis
2	30	Widespread	Psoriatic arthropathy	2	S.A.	Insufficient	
3	58	Generalized	Emphysema, cardiac insufficiency	6	S.A.	Good	Intercurrent relapse; pulmonary embolism
4	42	Generalized	No	3½	S.A.	Good	Cheilitis
5	60	Generalized	Cardiac insufficiency, hypertension	3½	S.A.	Very good	Nausea, vomiting (digitalization); elevated ESR
6	81	Generalized	Cardiac insufficiency, asthma	4	S.A.	Moderate	Intercurrent relapse; cheilitis
7	56	Widespread	No	6	3 weeks S.A. 3 weeks D	Good	Paronychia
8	40	Erythroderma; pustular	Psoriatic arthropathy	6 6	3 weeks S.A. 3 weeks D	Good	Dry lips
9	31	Generalized	No	5‡	3 weeks S.A. 2 weeks D	Very good	Elevation of SGOT (36 IU) and SLDH (325 IU)
10	21	Generalized	No	4	2 weeks S.A. 2 weeks D	Very good	Cheilitis
11	53	Widespread	No	3	D	Very good	
12	66	Widespread	Cirrhosis, ascites	3	2 weeks S.A. 1 week D	Very good	Dry lips

*S.A. = 2% salicylic acid in petrolatum; D = 0·1% dithranol + 2% salicylic acid in petrolatum.

† Assessment of the clinical response was as follows: *very good* = full remission (nearly all lesions cleared); *good* = remission (over 75% of the lesions flattened); *moderate* = less than 75% of lesions improved.

‡ Dose 125 mg/day.

concentrations of dithranol (0·1% dithranol+2·0% salicylic acid in petrolatum). The retinoid was administered orally, 75–100 mg/day, i.e. approx. 1 mg/kg body weight, one capsule of 25 mg with breakfast, lunch and dinner.

The *results* are shown in Table 1. Before and at the end of the oral treatment laboratory controls were made, including ESR, peripheral blood and urine status, platelets, blood urea and creatinine levels, serum electrophoresis, transaminases, LDH, alkaline phosphatase and blood sugar. None of these examinations showed significant changes except in one patient who had a slight elevation of SGOT and LDH after 125 mg daily for 6 weeks. The most prominent *side-effects* we found were cheilitis (× 4), dry lips (× 2) and paronychia (× 2). One patient experienced some nausea and vomiting; however, he also received digitalis preparations during his hospitalization because of cardiac insufficiency. Another patient had a pulmonary embolism, but we did not consider this to be related to the drug; the medication was continued, the skin lesions responded excellently and he left the hospital on dicoumarins in good condition.

Summarizing our present experience we feel that the new retinoid signifies some progress in the systemic treatment of psoriasis. Given orally in severe generalized cases it may replace methotrexate and similar cytotoxic drugs. Furthermore, its combined application with low concentrations of dithranol seems to increase the clinical response and to shorten the duration of the traditional local treatment. While this clinical trial was taking place corresponding observations were made by Schimpf (1976) and Landes (personal communication). In the light of these stimulating preliminary results we intend to strengthen our common efforts and to find the best modalities of application. A team from several Departments is now involved in further investigations with the new retinoid in psoriasis.

REFERENCES

Bollag, W. (1974a) Antitumor effect of a new retinoid acid analog. *Experientia (Basel)*, **30**, 1198.

Bollag, W. (1974b) Therapeutic effects of an aromatic retinoic acid analog on chemically induced papillomas and carcinomas in mice. *European Journal of Cancer*, **10**, 731.

Ott, F. & Bollag, W. (1975) Therapie der Psoriasis mit einem oral wirksamen neuen Vitamin A-Säure Derivat. *Schweizerische medizinische Wochenscrift*, **105**, 439.

Orfanos, C.E., Schmidt, H.W., Mahrle, G. Gartmann, H. & Lever, W.F. (1973) Retinoic acid in psoriasis: Its value for topical therapy with and without corticosteroids. *British Journal of Dermatology*, **88**, 167.

Rünne, U., Orfanos, C.E. & Gartmann, H. (1973) Perorale Applikation zweier Derivate der Vitamin A-Säure zur internen Psoriasis-Therapie. 13-cis-beta-Vitamin A-Säure und Vitamin A-Säure aethylamid. *Archiv für Dermatologische Forschunge*, **247**, 171.

Schimpf, A. (1976) *Zeitschrift für Haut- und Geschlechtskrankheiten*, in press.

British Journal of Dermatology (1976) 95, 105.

Correspondence

IS ACTINIC (SOLAR) DAMAGE THE PROVOKING CAUSE OF 'POST-INFLAMMATORY ELASTOLYSIS AND CUTIS LAXA (PECL)'?

SIR, on reading the paper by Verhagen & Woerdeman (1975) I noticed an apparent similarity of PECL to certain reactions occurring in Australia which may be solar-induced and for which I have suggested the name actinic granuloma (O'Brien, 1975).

The chief points of similarity between PECL and actinic granuloma are the preference for the sun-exposed areas, the sequence of papules, plaques and rings, and the subsequent local atrophy along with a microscopic reduction in elastic fibres. Both conditions appear to display clinical and histological evidence of diffuse actinic elastosis (solar or 'senile' degeneration of the dermis) and indeed this background seems to be prerequisite for the focal lesions.

Admittedly there are some uncertainties, the most significant of which concerns the histological picture of PECL. Thus Verhagen & Woerdeman do not state that their dermal 'degeneration' is actinic elastosis (that is, the alteration of dermal fibres to take the blue stain of haematoxylin). Nor do they specifically relate the granulomatous reaction of PECL to a resorption of the elastotic fibres in a zonal manner as described for actinic granuloma. However, their entire description seems very suggestive of both of these basic features.

In an apparently similar case of cutis laxa (affecting a fireman, a possibly significant circumstance) McCarthy, Warin & Read (1965) describe a foreign-body giant cell reaction around fibres which, judged from the description, were probably actinically damaged or elastotic (the elastic fibres showed 'clumping' and 'fragmentation', the collagen was 'irregular and slightly swollen').

Having noted in passing that 'sunlight' might be a factor in PECL, Verhagen & Woerdeman then go on to suggest insect bites as the basis of the focal lesions. They comment that PECL commences about the time the infants 'start to play in grass and vegetation'. However, this is a circumstance which implies a greater degree of solar exposure.

Verhagen & Woerdeman do not refer to granuloma multiforme (Leiker, Kok & Spaas, 1964), a disease of adult African Negroes which I believe (O'Brien, 1975) is also akin to both PECL and actinic granuloma.

If actinic radiation is indeed the immediate cause of PECL, the extreme susceptibility of the affected female infants requires explanation. For this we need to assume a pronounced genetic defect. In Australia some observers believe that genetic factors are also important in actinic granuloma and that persons of Celtic origin are more prone than those of other origin. However, nothing matching the susceptibility of PECL patients has been described in Australia.

Should further investigation establish that PECL is actinically induced, support will thereby be given to the propositions that persons with black (*light-resistant*) skin are not as immune to actinic damage as is commonly thought and, further, that deeply penetrating infra-red (heat) rays rather than light rays are probably responsible (O'Brien, 1975).

In summary, it is proposed that PECL, granuloma multiforme and actinic granuloma are related and that infra-red radiation may be the common provocative external factor. The degree of genetic predisposition seems to vary greatly between the three disorders. In future studies, particular attention needs to be given to the antigenic and enzymatic aspects of the elastolysis.

Pathology Laboratory,
Medical Centre,
66 High Street,
Randwick, 2031,
Sydney, Australia

JOHN P. O'BRIEN

REFERENCES

LEIKER, D.L., KOK, S.H. & SPAAS, J.A.J. (1964) Granuloma multiforme. *International Journal of Leprosy*, **32**, 368.

MCCARTHY, C.F., WARIN, R.P. & READ, A.E.A. (1965) Loose skin (Cutis Laxa) associated with systemic abnormalities. *Archives of Internal Medicine*, **115**, 62.

O'BRIEN, J.P. (1975) Actinic granuloma. *Archives of Dermatology*, **111**, 460.

VERHAGEN, A.R. & WOERDEMAN, M.J. (1975) Post-inflammatory elastolysis and cutis laxa. *British Journal of Dermatology*, **92**, 183.

CAUSATION OF PSORIATIC EPITHELIAL HYPERPROLIFERATION

SIR, Shahrad & Marks (1976) recently reported, in psoriatics, significantly elevated labelling indices of the interfollicular epidermis and its morphological extension, the *upper* external root sheath. In these same patients they reported normal labelling indices of the matrix and external root sheath (presumably below the level of the isthmus). On the basis of these data, they manage to find support for the notion of dermal causation of psoriatic epithelial hyperproliferation.

It seems to me that their data are more suggestive of the opposite view, namely that psoriasis represents defect(s) in epidermal differentiation. It is well known, from the work of Hermann Pinkus, that differentiation, and in particular keratinization, of epidermis differs from that of hair. The hair product itself and the linings of the inner root sheath keratinize at different levels and in different ways. Moreover, the process of keratinization of the external root sheath differs depending on whether the sheath is examined below, at or above the isthmus. In view of these normally occurring differences in keratinization, it comes as no surprise that labelling indices differ as well.

Labelling differences between normals and psoriatics are specifically limited to the tissue affected by psoriasis—which is epidermal epithelium, not hair epithelia. It is suggested that psoriasis involves defect(s) in differentiation of epidermal epithelium without involving defect(s) in the quite unrelated and diverse paths of differentiation seen in hair epithelia. Dermal mechanisms need not be invoked.

Serramonte Medical and
Dental Center,
1500 Southgate Avenue,
Daly City,
California, U.S.A.

DAVID PASLIN

REFERENCE

SHAHRAD, P. & MARKS, R. (1976) Hair follicle kinetics in psoriasis. *British Journal of Dermatology*, **94**, 7.

Book Reviews

Practical Dermatology. I.B.SNEDDON and R.E.CHURCH (1976) 3rd edn. London: Edward Arnold. Pp. 226. Price £7.00.

This slim hard-backed introduction to clinical dermatology was an instant success when it first appeared in 1964. The new third edition has been revised but not enlarged and its remains the best of the small books for the undergraduate.

As before, attention is focussed firmly on the clinic and the commonest disorders seen there. Thus only 4 pages are devoted to the function and structure of skin; a parsimony requiring every word in this chapter to be chosen with care. It is, therefore, a pity to see that this brief account contains several inaccurate statements such as 'the epidermis made up of many layers of horny plates' (this surely refers to the stratum corneum) or 'the presence of pigment *in the lower layers* of the epidermis filters out . . . ultraviolet radiation' (stratum corneum pigment is mainly responsible). Again, 'the prickle cells are held together by inter cellular bridges' is a misconception from pre electron-microscope days, which is not dispelled by the subsequent reference to electron microscopic findings.

The brief accounts of common diseases are excellent and the authors resist the temptation to try to pack in facts at the expense of readability. Nevertheless, more information could sometimes be given without sacrificing brevity or style. Thus the reader is properly warned against continued use of antimalarials in lupus erythematosus 'in view of their side-effects' (p. 34); here an additional word or two would have pinpointed retinopathy as the important hazard.

Now that the excellent Wolfe Atlas by Levene and Calnan is available one may question the usefulness of some of the black and white photographs, although many are remarkably effective. Possibly their space would be better devoted to more text with a few pages of colour photographs of conditions where hue is all important in diagnosis. Doubtless, such changes would be impossible without increasing the price beyond the undergraduate's pocket.

However, these are carping criticisms of a valuable and successful little book which I for one will continue to recommend as the introduction of choice to clinical dermatology for the undergraduate. Its continued popularity with students will be ensured by this new edition.

H.Baker

Clinics in Rheumatic Diseases. Vol. 1, no. 3 (December 1975) Systemic Lupus Erythematosus. Edited by Naomi F.Rothfield. London: W.B.Saunders.

This is a most useful volume covering subjects as varied as animal models for systemic lupus erythematosus (SLE) to cytotoxic agents in its management. The contributions are concise and well documented.

One chapter underlines the increasing recognition of the importance of neuropsychiatric abnormalities in SLE. The author stresses the need for evaluation of neurological and psychiatric function in all cases and also for randomized trials to assess treatment. Neurological disease occurs late in SLE, unlike renal involvement which usually occurs early, but the mortality is about the same.

Another chapter deals with SLE and pregnancy. The course of SLE is most favourable when the condition is quiet at the beginning of pregnancy. Exacerbations in pregnancy are treated best by increasing the dose of corticosteroids, rather than by abortion. There is little evidence that the fetus will be adversely affected by steroids, although the mother should not breast feed.

Other contributions deal with cell mediated immunity, the role of viruses and drugs, anti-nucleic acid antibodies and complement. Lowered complement levels are particularly useful in the diagnosis and management of nephritis but they must always be considered in conjunction with other clinical and laboratory features.

The author of the chapter on cytotoxic agents concludes that these drugs should be used only in patients with the poorest prognosis. This needs substantiating by further controlled trials.

This book is highly recommended.

N.R.Rowell

Control of Hospital Infection: A Practical Handbook. E.J.L.Lowbury, G.A.J.Ayliffe, A.M. Geddes and J.D.Williams (1975) London: Chapman and Hall. Pp. 306+xi. Price £8.50.

This is a very good 'How to' book. Theory is kept to a minimum and the principal parts are assessments of the efficacy and comparative costs of a variety of procedures for cleaning wards or isolating and treating patients. The authors base their recommendations on procedures tested in the West Midlands Region.

In a short section specific to Dermatological Wards the authors suggest wards should be kept solely for patients with skin disease or failing that one understands that patients with desquamating lesions, herpes simplex or zoster, scabies or generalized eczema should be kept in isolation in single rooms for the duration of their illness. Those with septic varicose ulcers should be treated in the same way.

Whilst it is to be hoped that most clinicians would be aware of differing costs in antibiotic therapy (Penicillin V: Orbenin: Carbenicillin in the ratio 1: 36: 516) it may be surprising to find skin degerming agent costs in the ratio of 1:8 for products often considered equivalent (chlorhexidine in alcohol: povidone iodine in alcohol).

The authors claim that the book is 'addressed to doctors, nurses, physiotherapists, radiographers and others who are involved in the treatment and care of patients, and in part also to administrators, architects, engineers, domestic superintendents and others whose work may influence the chances of infection among patients and staff.' It seems certain that an institution which cares abouts its patients will have a very well thumbed copy (or copies!) of this book.

W.E.NOBLE

Drugs and Disease. Symposium Organized by the Royal College of Pathologists delivered in London in February 1975. Edited by SHEILA WORLLEDGE (1975) Published for the Royal College of Pathologists by the *Journal of Clinical Pathology*, **28,** Suppl. (Roy. Coll. Path.), **9,** 1–124. Price £3.00.

This volume will interest a wide variety of readers. It concerns effects and ill effects of a number of drugs (and some poisons) on each other and on a selection of organs and diseases. Each of the 20 chapters is contributed by well known author(s) but necessarily their contributions cover only selected aspects. Notable is W.D.M. Paton's 'Molecular Basis of Drug Toxicity' which discusses lipid-soluble substances and the cell membrane and thoughtfully hints at the implications to all of us who live in this complicated chemical environment, contaminated by many fat soluble substances such as petrol fumes. The two papers on Drugs and the Skin (Dr Sneddon and Professor Turk) are both admirable, reflect our Specialties' contributions and importance in this sphere, and emphasize that the diagnosis of 'drug rash' may be difficult to substantiate and be non-immunological in pathogenesis.

P.W.M.COPEMAN

Oral Mucosa in Health and Disease. Edited by A.E.DOLBY (1976) Oxford: Blackwell Scientific Publications. Pp. 528, 72 illustrations. Price £16.00.

This book consists of a series of essays written by research workers mainly in the field of oral pathology and the title should not mislead prospective readers that it is clinically orientated.

The first chapter of 120 pages, though entitled 'the structure and function of normal human oral mucosa', compares skin and mucosa. Much of the original work quoted applies to the skin and will be familiar to dermatologists.

After a short account of salivary function there is a detailed chapter on immunoglobulins, only the latter half of the chapter applying to immunoglobulins of the oral cavity.

Professor Jones attempts the difficult task of describing the oral markers of internal disease and his contribution is so compressed that the result is superficial and scrappy. There are a number of factual errors, for instance it is only sufferers from recessive congenital erythropoietic protoporphyria who show pink teeth, not the usual dominant variety.

The five lines devoted to acrodermatitis enteropathica do not include a reference to the discovery that it responds to zinc, nor is this mentioned in the following chapter which deals in fact with the metabolism of zinc. The original papers on this subject were published in 1973.

The two chapters on malignancy and premalignant changes are clearly written and underline the importance of tobacco as a carcinogen.

Professor Dolby reviews the immunology of oral ulceration including pemphigus but again there is no reference to the immunological discovery that benign mucous membrane pemphigoid does show antibodies, which was published in 1973.

The best of this book is kept to the end. There is a beautifully illustrated account of Sjögren's syndrome from the Glasgow department and the account of candidal infection of the mouth from Cardiff is informative and succinct.

It is difficult to see who will use this book; there is little for the clinician and it is disappointingly not up-to-date enough for the research worker.

I.B.SNEDDON

The Molecular Biology of Skin. PAUL D.MIER and DENIS W.K.COTTON (1976) Oxford: Blackwell Scientific Publications. Pp. 449. Price £14.50.

Since Rothman's 'Biochemistry of Skin' is now well out of date, there is a need for a book on this topic. This book goes part of the way towards filling this gap but suffers in comparison. Its great virtue is that it contains a vast amount of biochemical information and an extensive bibliography which could be of great help to anyone trying to find their way into the literature. Much of the biochemistry is straightforward, marginally related to skin. Several recent books have contained up-to-date information on, for example, collagen and mucopolysaccharide metabolism.

This book is presented in three sections. The first—'The normal skin'—is concerned with chapters on the various chemical classes, e.g. lipids, polysaccharides, structural protein, etc. and there is a strong emphasis on metabolism and enzyme function. I felt that the section on pigment did not do justice to the importance of the subject but other sections were detailed and adequate. This section occupied half the book and the remaining half was split equally between the 'Experimental Pathology of the skin' and 'Diseases of the skin'. Each of these sections commences with a chapter on 'Orientation' with the objective of reconciling the divergent skills and knowledge of the biochemist and the physician. In my opinion this interesting idea was not successful and the chapters offered too often some homespun philosophy and trite homilies, *sic* 'Skin like other tissues has evolved over a long period'.

I found the 100 pages or so on 'Experimental Pathology' difficult to read. It is very condensed and the pattern of presentation was essentially the effects of physical and chemical agents on the various enzyme systems and the chemical classes which are discussed in Section 1.

The last section was very disappointing. The dermatological chapters are physically separated from the preceding biochemistry and thus it is difficult to make correlations of clinical and biochemical abnormalities. It also necessitates some repetition. This dichotomy is quite outstanding in relation to lipid metabolism and acne. In the latter chapter, the possible role of squalene in its pathogenesis is not even considered. The authors minimize any immunological data (although they do provide the key references). In a book entitled 'Molecular Biology' as opposed to 'Biochemistry' there is no justification for this.

This is very nearly an important book and worthy of expansion in the area of biochemistry in relation to disease processes.

C.N.D.CRUICKSHANK

British Journal of Dermatology (1976) **95**, 111.

Review

The clinical importance of 'weathering' in human hair

The term 'weathering' as applied to hair is familiar to the textile scientist (Robbins, 1967) and to the cosmetic chemist (Brown & Swift, 1975), but much less so to the dermatologist. Yet weathering, a term which conveniently describes the cumulative effect of climatic exposure on the chemical and physical structure of the hair shaft, is a factor in many clinical syndromes in the dermatological department. It may be solely responsible for grossly evident abnormalities, but produces its more striking effects in combination with cosmetic procedures, or when acting on hair which is already defective.

The clinical importance of weathering has been greatly increased by the recent popularity of wearing the hair long. The duration of anagen, which is probably genetically determined, ranges from 3 to 6 years, and the length of uncut hairs from 50 to 80 cm. With the scanning electron microscope (SEM) it has been shown (Bottoms, Wyatt & Comaish, 1972) that apparently normal hair shafts show a progressive loss of cuticular scale pattern from root to tip. In man it is difficult to differentiate the effect of weathering alone from the combined effect of weathering and trauma inflicted for cosmetic purposes, since even the more uncouth seldom refrain completely from the use of a brush and comb. Even combing adds to the erosion of cuticular scales, the margins of which are gradually chipped away to leave the cortex more or less exposed. Variation in the degree of cuticular erosion in different areas of the scalp could be related to combing habits (Swift & Brown, 1972). In a recent investigation (Brown & Swift, 1975) of hair at least 50 cm long (and therefore, at its tip, exposed to weathering for at least 3 years) it was shown that the hair near the root fractured in a strikingly different fashion from the same hair near its tip. Segments of hair were extended in a tensile tester until they fractured, and the fractured surfaces were then examined in the SEM. Near the root end the hair showed a clear transverse fracture, 'as if cut with a knife'. Near the tip of extremely weathered hairs the fracture was ragged, the cortex separating into fibrillae, with longitudinal splits extending back along the shaft. In intermediate segments the type of fracture was as expected again related to the degree of weathering. Combing experiments carried out in the SEM showed that tangled hairs, forming tight bands and loops, fracture in a similar fashion.

The changes produced by cold-waving solutions or by the application of excessive heat in waving or dyeing have been carefully studied, since they are often readily detectable with the light microscope (Braun, 1963). In the SEM (Orfanos & Mahrle, 1971) such insults, usually in combination with the mechanical trauma of brushing and combing, have been shown to produce fracture and detachment of free edges of cuticular cells, the pattern of which becomes disorderly. With increased or repeated injury the cuticular cells separate, exposing the cortex. Such changes obviously are more marked as the distance from the scalp increases, since such hair has had greater exposure to weathering as well as to previous cosmetic procedures, and although they necessarily resemble in general those attributed to weathering alone, extensive exposure to individual chemical or physical agents may produce different and distinctive changes (Orfanos & Mahrle, 1971).

An important factor in the weathering process is exposure to sunlight, which causes some degradation of hair keratins and a loss of tensile strength (Beyak, Kass & Meyer, 1971). The degree of damage correlates with the total radiation received, rather than with the character of this radiation. The deleterious effect of sunlight is well illustrated by the seasonal recurrence of trichorrhexis reported by Papa, Mills & Hanshaw (1962). The fractures induced experimentally in weathered hair (Brown & Swift, 1975) have many features in common with trichorrhexis nodosa.

This short review may serve to introduce the dermatologist to a very large literature in the journals of cosmetic science and textile technology. The clinical lesions are of considerable practical importance. Normal hair, if it is long, may be damaged sufficiently by the combination of cosmetic procedures even when properly applied, and natural weathering, for it to show such defects as 'split ends' and 'brittleness' and 'dryness', for which the patient may seek medical advice. In such cases segments of hair near the scalp and segments cut from the tips should be examined microscopically; even the light microscope shows distinctive changes. Congenitally abnormal hair tends to show even greater susceptibility to weathering and to cosmetic trauma. Such cases include such well characterized defects as monilethrix and pseudo-monilethrix (Bentley-Phillips & Bayles, 1973) and those children with short brittle hair which shows no evident structural abnormality under the SEM. The reduction of hairdressing trauma of all kinds and the avoidance of unnecessary sun exposure may allow the hair to grow to a cosmetically more acceptable length in patients to whom the dermatologist can at present offer nothing other than this advice.

Addenbrooke's Hospital,
Cambridge

ARTHUR ROOK

REFERENCES

BENTLEY-PHILLIPS, B. & BAYLES, M.A.H. (1973) A previously undescribed hair anomaly (pseudo-monilethrix). *British Journal of Dermatology*, **89**, 159.

BEYAK, R., KASS, G.S. & MEYER, C.F. (1971) Elasticity and tensile properties of human hair. II. Light radiation effects. *Journal of the Society of Cosmetic Chemists*, **22**, 667.

BOTTOMS, E., WYATT, E. & COMAISH, S. (1972) Progressive changes in cuticular patterns along the shafts of human hair as seen by scanning electronmicroscopy. *British Journal of Dermatology*, **86**, 375.

BRAUN, W. (1963) Morphologische Veränderungen der menschlichen Kopfhaares mit Dauerwell-Schäden. *Hautarzt*, **14**, 468.

BROWN, A.C. & SWIFT, J.A. (1975) Hair breakage: the scanning electron microscope as a diagnostic tool. *Journal of the Society of Cosmetic Chemists*, **26**, 288.

ORFANOS, C.E. & MAHRLE, G. (1971) Human hair and how it changes under cosmetic procedures *in vivo*. *Parfumerie und Kosmetik*, **52**, 203 and 235.

PAPA, C.M., MILLS, O.H. & HANSHAW, W. (1962) Seasonal trichorrhexis nodosa: role of cumulative damage in frayed hair. *Archives of Dermatology*, **106**, 888.

ROBBINS, C.R. (1967) Weathering in human hair. *Textile Research Journal*, **40**, 891.

SWIFT, J.A. & BROWN, A.C. (1972) The critical determination of fine changes in the surface architecture of human hair due to cosmetic treatment. *Journal of the Society of Cosmetic Chemists*, **23**, 695.

British Journal of Dermatology (1976) **95**, 113.

News and Notices

VIIIth World Congress on Prevention of Occupational Accidents and Diseases

The VIIIth World Congress on the Prevention of Occupational Accidents and Diseases will be held in Bucharest (Romania) from 17 to 21 May 1977.

Information may be obtained from: National Organising Committee, VIIIth World Congress on the Prevention of Occupational Accidents and Diseases, Ministry of Labour, 1–3 Strada Scaune, Bucharest, Romania.

Alfred–Marchionini prize 1977

On the occasion of the XV International Congress of Dermatology in Mexico the Alfred-Marchionini-Prize founded in 1966 will again be awarded.

For the Alfred Marchionini Research Allowance—the second part of this prize—proposals are requested before the 31 December 1976, to the Chairman of the Board of the Alfred-Marchionini-Foundation, Professor Dr med. Th. Nasemann, Director of the Dermatological Hospital of the University of Frankfurt, D 6000 Frankfurt, Theodor-Stern-Kai 7, or to the Secretariate of the Alfred-Marchionini-Foundation, D2057 Reinbek near Hamburg, Danziger Str. 5, West Germany.

Medical directors of hospitals, research or other scientific institutes, are entitled to submit proposals on behalf of young scientists in the fields of Medicine, Biochemistry or Biopharmacy. In accordance with the interest of Alfred Marchionini in international human relations, the relevance to international dermatology, as well as the merit of the scientific work, will be considered. Proposals must include a detailed appreciation of the candidate. The Alfred Marchionini Prize 1977 is endowed with DM 10,000.

Spanish Academy of Dermatology Prizes

The Sainz de Aja Prize (15,000 Pesetas) is open to doctors from Spain and Ibero-Latin Countries. The Spanish Academy of Dermatology Prize (15,000 Pesetas) is open Internationally. Both will be awarded for original papers on any aspect of Dermatology or Venereology. The paper (three copies, with the name and address of the author in a separate envelope) should be sent to: The Academia Espanola de Dermatologia, Calle Sandoval 7, Madrid, before the 1 December 1976. The winning papers will be kept at the Academy and will be published in *Actas Dermosifiliográficas.*

European Society for Dermatological Research

The second clinically orientated symposium of the E.S.D.R. will be held in Cardiff on the 14 January 1977. The subject will be 'The Ichthyoses'

Further details can be obtained from either Professor Rugero Caputo, Clinica Dermatologica, Universita Di Milano, 20122 Milano, Italy, or from Dr R. Marks, Welsh National School of Medicine, Heath Park, Cardiff, CF4 4XN. Anyone wishing to read a paper at this meeting should contact one of the above before the 1 October 1976.

Prostaglandins and Cyclic Nucleotides: Dermatological Aspects. Department of Dermatology, Hvidovre Hospital, University of Copenhagen. Friday 24 September 1976

This is the first of a series of basic workshops in various areas of investigative dermatology and sponsored by the ESDR. The workshop will take place at 'Rungstedgaard' Congress center in Copenhagen. The participants will have one full day for the discussions. Accomodation for two nights and meals will be covered. The number of participants (active contributors) will be limited to 20. Applicants are kindly asked to supply information on their contribution and fields of interest as soon after 1 August 1976 as possible.

Anyone wishing to contribute to this meeting should contact either: Professor Malcolm W. Greaves, The Institute of Dermatology, St. John's Hospital for Diseases of the skin, Lisle Street, Leicester Square, London, WC2H 7BJ, or Professor Jørgen Søndergaard, Department of Dermatology, Hvidovre Hospital, Kettegaard alle 30, DK-2650 Hvidovre, Copenhagen.

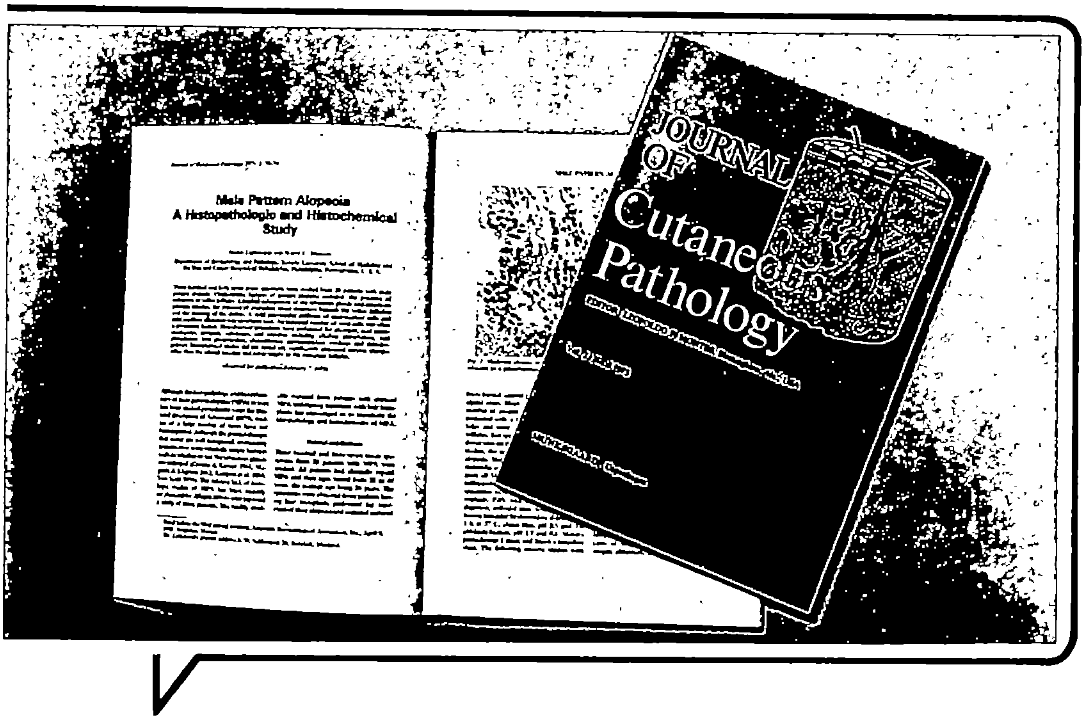
Annual Meeting of ESDR

The 1977 Annual Meeting of the European Society for Dermatological Research will take place in the Rai Congrescentrum in Amsterdam on 2–4 May 1977.

Attendance at the scientific sessions will be open to all members, contributors and guests. Non-members, who wish to present a paper are kindly requested to submit an abstract. They will be invited to attend the meeting as either contributors or guests.

Abstracts presented on the official form should be posted before 15 December. Abstract forms and information about the meeting can be obtained from: Ch. M. Lapiere, ESDR Secretary, Department of Dermatology, Hôpital de Bavière, B-4020 Liege, Belgium (tel. 041–43.93.72).

Dermatology


Male Pattern Alopecia
A Histopathologic and Histochemical Study
Journal of Cutaneous Pathology

Journal of Cutaneous Pathology

Editor: Leopoldo F. Montes, Birmingham, Alabama

Journal of Cutaneous Pathology publishes manuscripts of the highest scientific quality in the field of cutaneous pathology. It aims at promoting and maintaining communication between clinicians and research workers. Cutaneous pathology will be interpreted to mean diseases of the epidermis, dermis and cutaneous appendages.
Original scientific articles on diagnostic and experimental cutaneous pathology are invited. Clinico-pathological studies will also be considered for publication.

As a special offer to new subscribers to JOURNAL OF CUTANEOUS PATHOLOGY we can offer you volumes 1 and 2 (1974 & 1975) FREE as long as the stock lasts so you can have the journal right from the beginning.

MUNKSGAARD · COPENHAGEN

Order Form to be sent in an envelope to:

MUNKSGAARD
35, Nørre Søgade
DK-1370 Copenhagen K.
Denmark

JOURNAL OF CUTANEOUS PATHOLOGY

☐ Please enter my subscription to start with volume 3, 1976. Subscription price D.Kr. 320.00 plus postage D.Kr. 14.00 (US$ 56.50 incl.postage).

☐ Please send me volumes 1 and 2 FREE (special offer to new subscribers only).

☐ I enclose ______________ in payment.

Name: ______________________

Address: ______________________

TEXTBOOK OF DERMATOLOGY

Available again as a reprint of the Second Edition

Edited by Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. *Second Edition,* 1972 (*Reprinted* 1975). 2236 pages, 1100 illustrations. Two volumes. £48.00

The appearance of the second edition of this book comparatively soon after the first testifies the success with which it has fulfilled a need.... these two volumes are essential to the dermatologist; the trained dermatologist will need them for frequent reference, while for the trainee they should be the sheet-anchor of systematic study.' *British Journal of Dermatology*

'In their second edition the authors have expanded on the knowledge previously presented and have added several quite useful and appropriate sections. The textbook will continue to be a superb reference book which should be available to all dermatologists; it is admirable as a comprehensive reference, detailed, and well-written survey of the field of dermatology.' *The Journal of Investigative Dermatology*

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition,* 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents.*

Manuscripts should be sent to the Editor, Dr R. H. Champion, Aldenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, 81, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 95, Number 1, July 1976

CONTENTS

PAGE

Clinical and Laboratory Investigations

Leukotactic Properties of Soluble Substances in Psoriasis Scale 1
HACHIRO TAGAMI AND SHIGEO OFUJI

Carbohydrate Metabolism in Lichen Planus 9
N. J. LOWE, A. G. CUDWORTH, S. A. CLOUGH AND M. F. BULLEN

Excision Repair of DNA in some Photodermatoses 13
M. ICHIHASHI AND C. A. RAMSAY

A Follow-up Study of Patients with Recurrent Urticaria and Hypersensitivity to Aspirin, Benzoates and Azo Dyes 19
ANN-MARI ROS, LENNART JUHLIN AND GERD MICHAËLSSON

The Role of Divalent Cations in Epidermolysis 25
ROBERT L. DIMOND, KENT L. ERICKSON AND KIRK D. WUEPPER

Necropsy and Ultrastructural Findings in Histiocytic Medullary Reticulosis 35
J. PIÑOL-AGUADÉ, J. FERRANDO, J. M. TOMÁS, C. MIERAS AND J. PEYRI

Leucosis and Skin: Acute Lymphoid Immunoblastic Leukaemia 45
M. G. BERNENGO, A. ZINA AND G. ZINA

Hypersensitivity of Scleroderma Cutaneous Vascular Smooth Muscle to 5-hydroxytryptamine 51
R. K. WINKELMANN, MARC E. GOLDYNE AND RONALD L. LINSCHEID

The Prognosis of Systemic Sclerosis 57
N. R. ROWELL

An Auto-antibody Profile in Alopecia Totalis and Diffuse Alopecia 61
REBECCA E. I. COCHRAN, JOHN THOMSON AND RODERICK N. M. MACSWEEN

Immunological Demonstration of Multiple Esterases in Human Eccrine Sweat 67
W. P. HERRMANN AND J. HABBIG

Pharmacology and Treatment

Septrin in the Treatment of Dermatitis Cruris Pustulosa et Atrophicans 71
WITOLD K. JACYK

The Treatment of Psoriatic Nail Dystrophy with Intradermal Steroid Injections 75
R. D. G. PEACHEY, R. J. PYE AND R. R. M. HARMAN

High Dose Dapsone Toxicity 79
WALTER B. SHELLEY AND MANFRED I. GOLDWEIN

Ciclopirox: a New Topical Pyrodonium Antimycotic Agent. A Double-blind Study in Superficial Dermatomycoses 83
V. N. SEHGAL

Case Reports

Prurigo Nodularis and Gluten Enteropathy 89
A. W. MCKENZIE, D. G. STUBBING AND B. L. ELVY

Sarcoidosis in a Child, Presenting as an Erythroderma with Keratotic Spines and Palmar Pits 93
J. G. L. MORRISON

Brief Communications

Pemphigus-like Syndrome Induced by D-penicillamine 99
S. G. TAN AND N. R. ROWELL

Beta-carotene in the Treatment of Discoid Lupus Erythematosus 100
PETER C. H. NEWBOLD

Systemic Use of a New Retinoid With and Without Local Dithranol Treatment in Generalized Psoriasis 101
C. E. ORFANOS AND U. RUNNE

Correspondence

Is Actinic (Solar) Damage the Provoking Cause of 'Post-inflammatory Elastolysis and Cutis Laxa (PECL)'? 105
JOHN P. O'BRIEN

Causation of Psoriatic Epithelial Hyperproliferation 106
DAVID PASLIN

Book Reviews 106

Review

The Clinical Importance of 'Weathering' in Human Hair 111
A. J. ROOK

News and Notices 113

11.12.76

Volume 95, Number 2, August 1976

British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

British Journal of Dermatology

EDITOR

DR R. H. CHAMPION
Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

DR J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London
DR RONALD MARKS, Cardiff
DR P.D.MIER, Nijmegen
DR J.N.S.MITCHELL, Royal Air Force
DR W.C.NOBLE, London
DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS

DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 U.K. (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed in Great Britain at the Alden Press, Oxford

84%

# of plantar warts wiped out with Salactol.

Salactol is the most reliable, best documented form of treatment yet developed for warts.
The evidence for Salactol is impressive. In a double blind controlled trial[1] 84% of cases with plantar warts were cured within a 12 week period. Its value is further confirmed in papers[2,3] *where Salactol is actually recommended by name.*
And it's a British product through and through.
Reliability, convenience and efficacy make Salactol the most widely accepted preparation for the treatment of warts in the UK.
Full information on Salactol is available from:

Dermal Laboratories Limited
Tatmore Place, Gosmore, Hitchin
Hertfordshire SG4 7QR

Salactol contains salicylic acid and lactic acid in a flexible collodion base and is presented in a 10ml bottle (more than enough for a full course of treatment) with a special applicator.

References:
1. Bunney, Mary H. (1971), Practitioner, **207**, 197.
2. Pegum, J. S. (1972), Practitioner, **209**, 453.
3. Bunney, Mary H. (1974), Prescribers Journal, **14**, 118.

Microvascular Injury (Major Problems in Dermatology Vol. 7)

Terence John Ryan MA, BM, B.Chem (Oxon) FRCP, Consultant Dermatologist, Oxfordshire Area Health Authority (teaching).

This clinical work presents a thorough and unique investigation into the consequences of injury to the small blood vessels of the skin. Immune complexes and infective agents are described in detail, and several chapters are devoted to analysis of the patient's response to treatment.

£16.00 448pp Hardback August 1976 ISBN 0 7216 7858 0

Psychophysiological Aspects of Skin Disease (Major Problems in Dermatology Vol. 8)

Professor F. A. Whitlock, Dept. Psychological Medicine, Royal Brisbane Hospital, Australia.

Professor Whitlock has chosen an interdisciplinary approach to study the psychophysiological aspects of dermatological disorders. Though he stresses the multifactorial nature of skin disease and the psychosomatic complications associated with it, at no time does the author lose touch with important clinical considerations.

Approx £8.50 256pp Illustrated Sept. 1976 Cased.

The Acnes (Major Problems in Dermatology Vol. 6)

W. J. Cunliffe M.D., Consultant Dermatologist, Skin Dept.
J. A. Cotterill M. D., Consultant Dermatologist, Dermatology Dept. The General Infirmary, Great George St, Leeds.

The Acnes presents an up-to-date thorough review of the many forms of this condition. As well as the inclusion of many acne remedies, there's a detailed section on the authors' own methods of treatment.

£8.50 306pp Cloth 1976 ISBN 0 7216 2785 4

Write now to Colin Dann, Publicity Manager for your inspection copy, quoting ref. WBS 11. Our Freepost address is Holt-Saunders Ltd, Freepost EA86, Eastbourne, East Sussex, BN21 1BR.

W. B. Saunders Company Ltd

1 St. Anne's Road Eastbourne BN21 3UN

'OUTLOOK IN PSORIASIS'

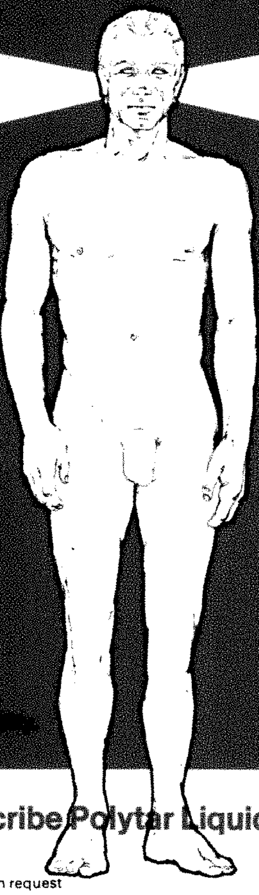
'The time-honoured applications of tar or dithranol and exposure to ultra-violet light remain the basis for local treatment'

Editorial, Brit. med. J. 1974,4,365

Polytar Liquid Psoriasis of the scalp

In a recent teaching hospital study, Polytar Liquid was compared with a standard tar-based formulation in 60 patients with psoriasis of the scalp. Polytar Liquid proved superior in the removal of scale and the relief of itchiness. Patient acceptability was excellent.

Polytar Liquid combines effective control with patient acceptability



Polytar Emollient Psoriasis of the body

[illegible]

"Polytar Emollient has a useful part to play in the treatment of psoriasis, particularly in out-patient treatment"

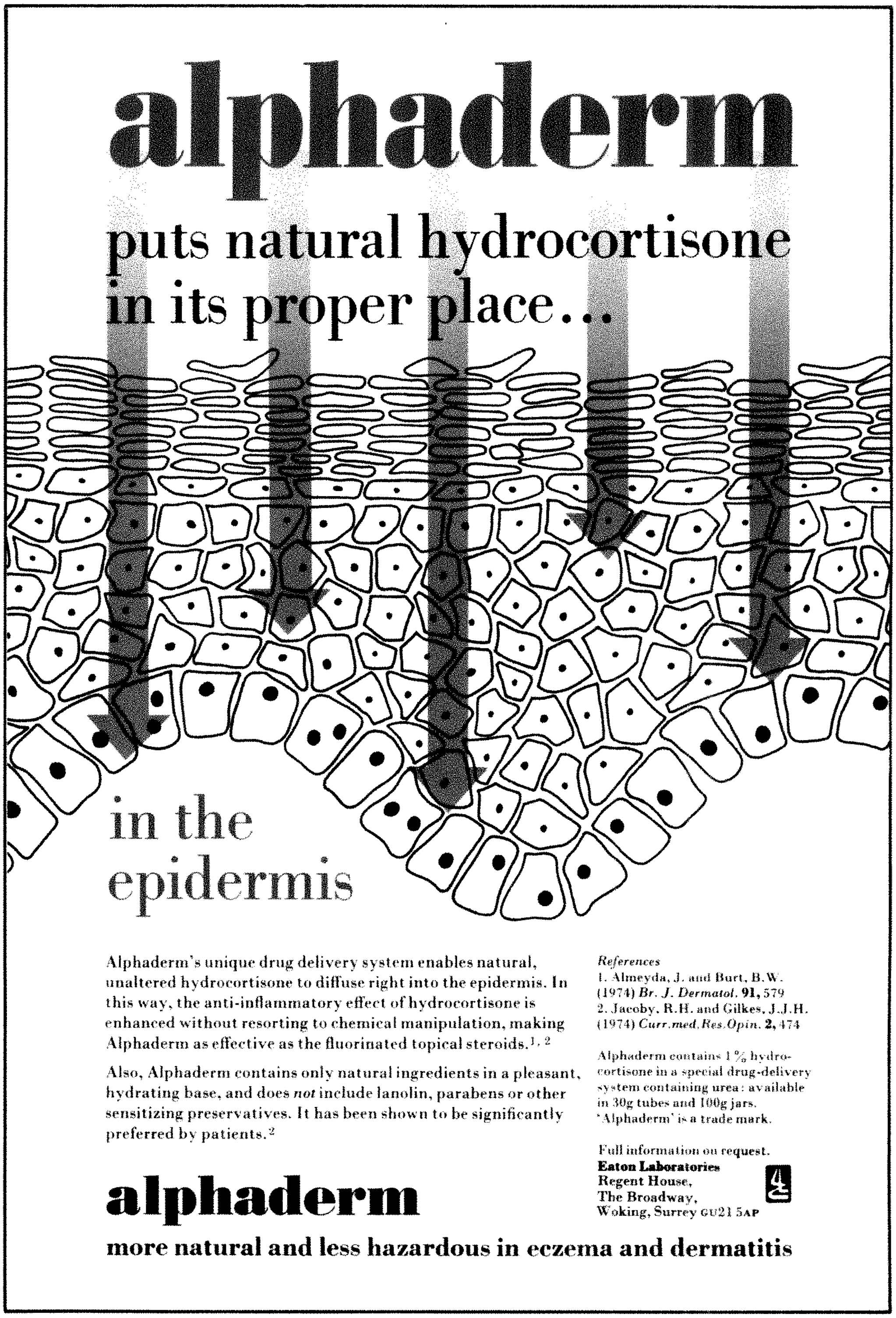
[illegible]

Polytar Emollient superior to liquor picis carbonis

For psoriasis – prescribe Polytar Liquid and Polytar Emollient

Full information on request

Stiefel Laboratories (UK) Limited 825 Yeovil Road, Slough, SL1 4JA



alphaderm

puts natural hydrocortisone in its proper place...

in the epidermis

Alphaderm's unique drug delivery system enables natural, unaltered hydrocortisone to diffuse right into the epidermis. In this way, the anti-inflammatory effect of hydrocortisone is enhanced without resorting to chemical manipulation, making Alphaderm as effective as the fluorinated topical steroids.[1, 2]

Also, Alphaderm contains only natural ingredients in a pleasant, hydrating base, and does *not* include lanolin, parabens or other sensitizing preservatives. It has been shown to be significantly preferred by patients.[2]

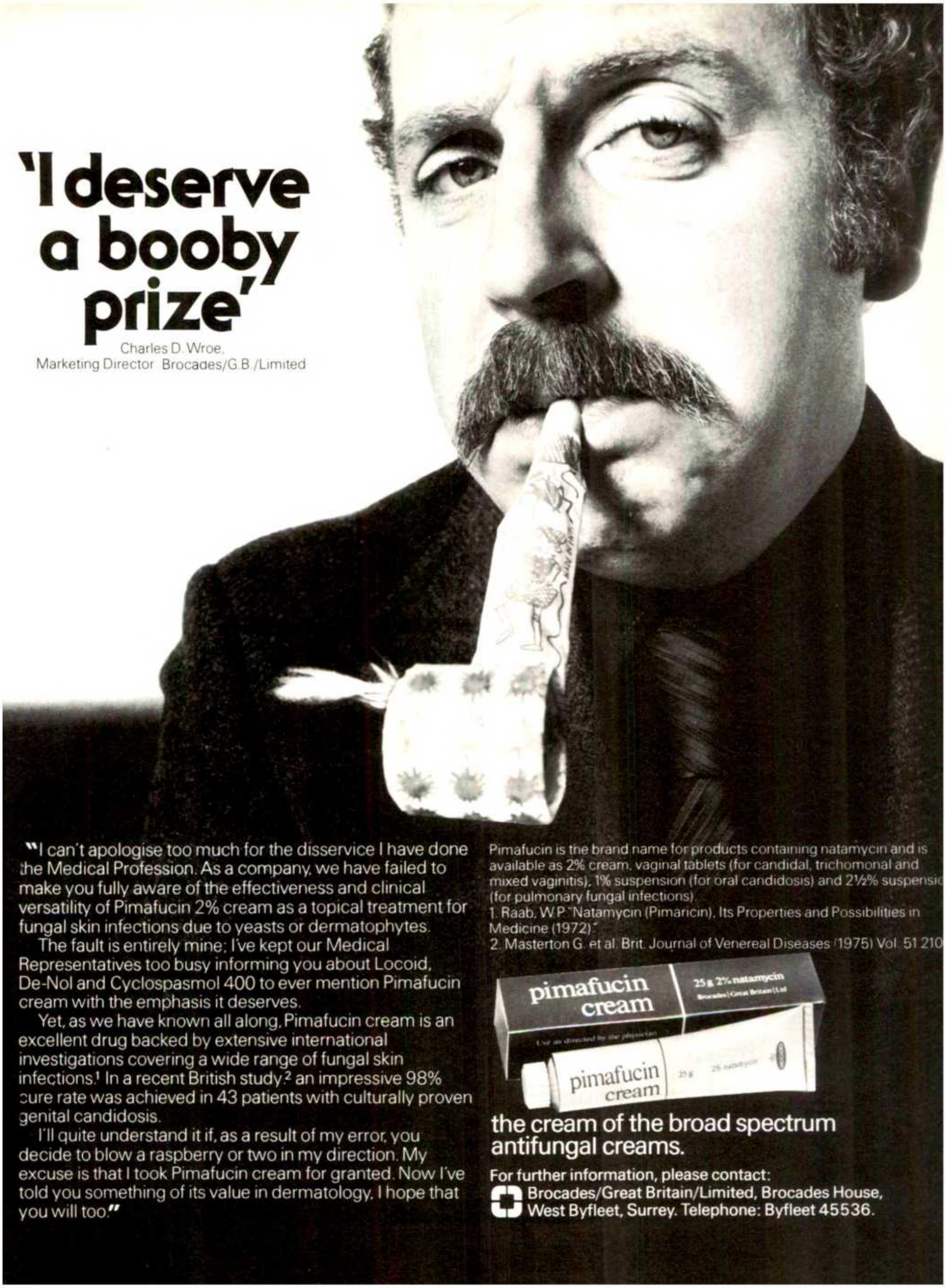
References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91,** 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr.med.Res.Opin.* **2,** 474

Alphaderm contains 1% hydrocortisone in a special drug-delivery system containing urea: available in 30g tubes and 100g jars. 'Alphaderm' is a trade mark.

Full information on request.
Eaton Laboratories
Regent House,
The Broadway,
Woking, Surrey GU21 5AP

alphaderm

more natural and less hazardous in eczema and dermatitis



'I deserve a booby prize'

Charles D. Wroe,
Marketing Director Brocades/G.B./Limited

"I can't apologise too much for the disservice I have done the Medical Profession. As a company, we have failed to make you fully aware of the effectiveness and clinical versatility of Pimafucin 2% cream as a topical treatment for fungal skin infections due to yeasts or dermatophytes.

The fault is entirely mine; I've kept our Medical Representatives too busy informing you about Locoid, De-Nol and Cyclospasmol 400 to ever mention Pimafucin cream with the emphasis it deserves.

Yet, as we have known all along, Pimafucin cream is an excellent drug backed by extensive international investigations covering a wide range of fungal skin infections.[1] In a recent British study,[2] an impressive 98% cure rate was achieved in 43 patients with culturally proven genital candidosis.

I'll quite understand it if, as a result of my error, you decide to blow a raspberry or two in my direction. My excuse is that I took Pimafucin cream for granted. Now I've told you something of its value in dermatology, I hope that you will too."

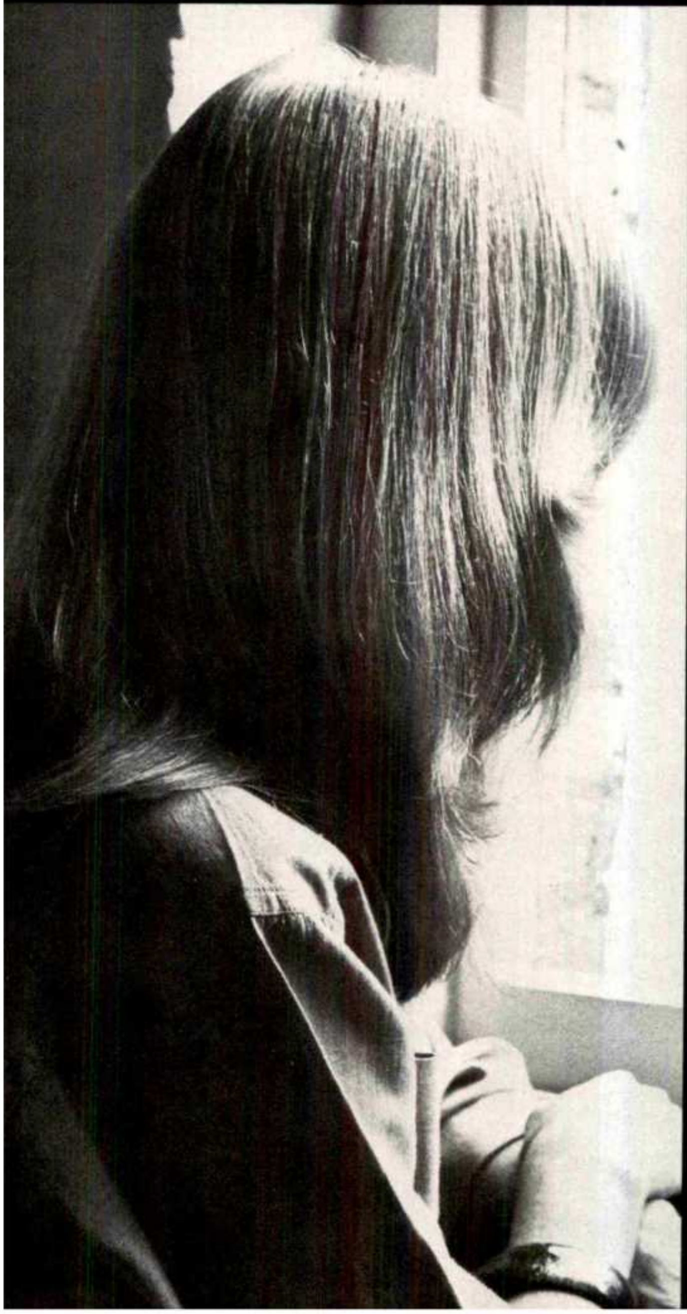
Pimafucin is the brand name for products containing natamycin and is available as 2% cream, vaginal tablets (for candidal, trichomonal and mixed vaginitis), 1% suspension (for oral candidosis) and 2½% suspensi (for pulmonary fungal infections).

1. Raab, W.P. "Natamycin (Pimaricin), Its Properties and Possibilities in Medicine (1972)."
2. Masterton G. et al. Brit. Journal of Venereal Diseases (1975) Vol. 51 210

the cream of the broad spectrum antifungal creams.

For further information, please contact:
Brocades/Great Britain/Limited, Brocades House,
West Byfleet, Surrey. Telephone: Byfleet 45536.

She's been to the chemist she's been to her doctor now she's come to you



In acne "... not all patients respond satisfactorily [to tetracycline] and there is a need for more effective or alternative therapy."

SEPTRIN* "... has been clinically effective in patients who did not respond to long-term tetracycline."

Brit J Derm. (1971), **84**, 366.

SEPTRIN FOR ACNE

Wellcome

Septrin contains trimethoprim and sulphamethoxazole. Full prescribing information is available on request.
Wellcome Medical Division
The Wellcome Foundation Ltd.
Berkhamsted, Herts

*Trade Mark

'The responsible carcinogenic wavelengths are probably located in the short sunburn waveband around 300nm'*

SPECTRABAN

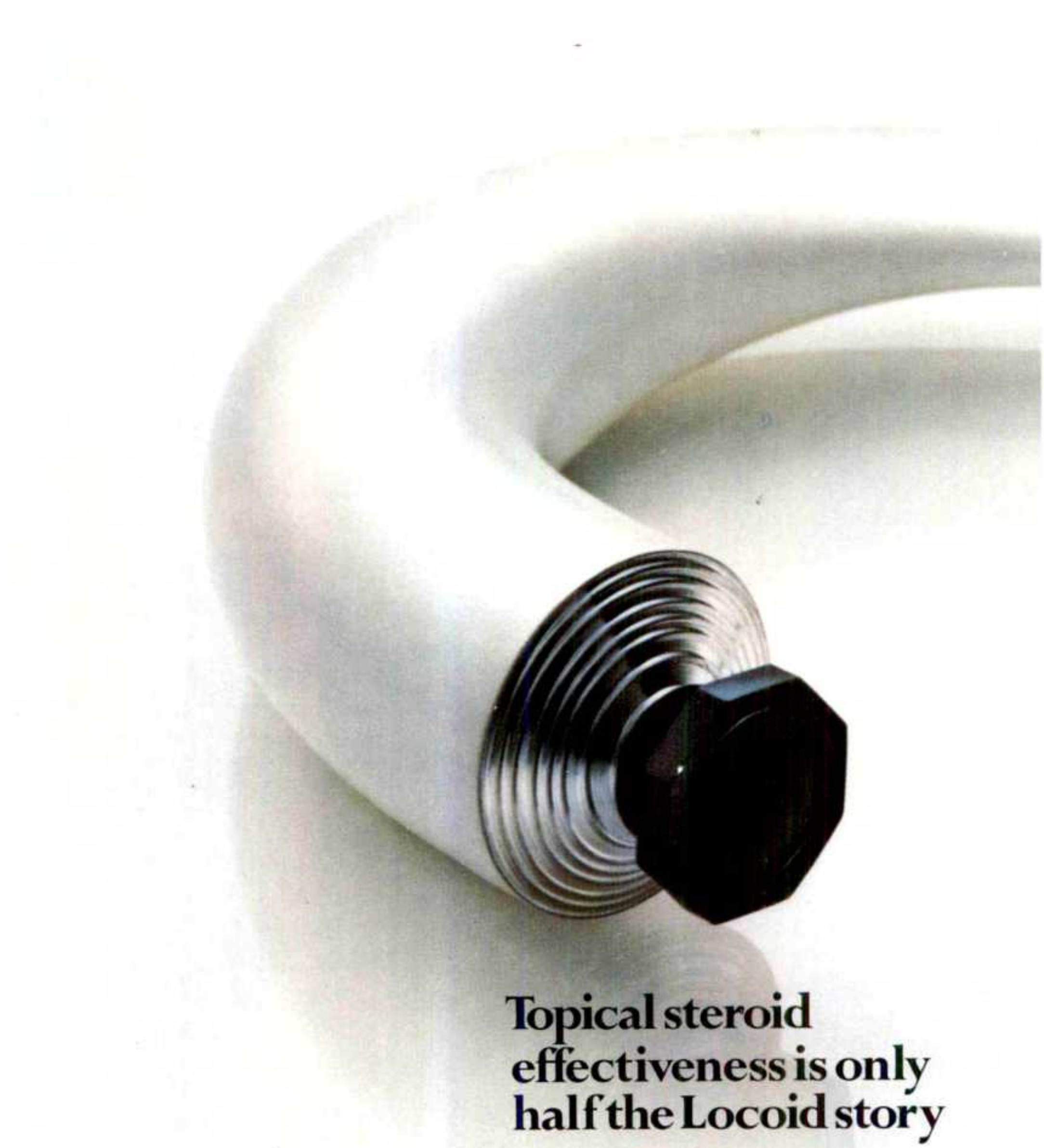
Provides complete protection against solar radiation within the range 290 - 320nm

Spectraban protects sun-sensitive skins

Spectraban: isoamyl – p – N, N – dimethylaminobenzoate 2.5% in ethyl alcohol.
Packs: 60ml bottles

* Extract from recorded lecture 'The Sun and the Skin' by a Consultant Dermatologist.

Full information on request
Stiefel Laboratories (UK) Limited 825 Yeovil Road, Slough, SL1 4JA



Topical steroid effectiveness is only half the Locoid story

...the other half is the relative freedom from topical side effects which are often encountered with the fluorinated steroids. Locoid offers the same level of success as these potent preparations–without fluorination.

locoid

the non-fluorinated way to topical steroid success

Locoid is a trade mark for hydrocortisone 17-butyrate.
Full information available on request:
Brocades (Great Britain) Ltd.,
Brocades House, West Byfleet, Surrey, KT14 6RA.
Telephone: Byfleet 45536.

British Journal of Dermatology (1976) **95,** 115.

Clinical and Laboratory Investigations

Kinetics of *in vitro* bovine keratohyalin synthesis

PETER M.ELIAS, WALTER G.KLINGLER AND PAUL M.MONTAGUE

Dermatology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Md., U.S.A.

Accepted for publication 12 December 1975

SUMMARY

A system is described for the study of the *in vitro* synthesis of bovine keratohyalin for periods of up to 24 h. Keratohyalin granules appeared morphologically unaltered during culture, although histochemical stains for RNA indicate a markedly diminished RNA content by 6 h. Incorporation of tritiated histidine began slowly, then became linear between 4 and 24 h in serum-containing media. However, following pre-incubation in serum-free media, increased incorporation occurred from time zero. The internal variation of the system using a standard 6 h pulse was ±10, ±24, ±22, ±18% in four separate experiments. Measurements of modulations in bovine keratohyalin synthesis *in vitro* may prove useful in the development of drugs of therapeutic potential in dermatology.

Bovine keratohyalin (KH) has been extensively studied by biochemical, histochemical, ultrastructural, and immunological techniques (Guss & Ugel, 1972; Ugel, 1971; Ugel & Idler, 1972). An *in vitro* system has been developed in order to study bovine KH synthesis in more detail (Elias, Montague & Ugel, 1972). Using the appropriate precursors, KH granules were labelled and subsequently studied both by autoradiography and by fractionation of labelled oligomers on polyacrylamide gels. These studies indicated that bovine KH is synthesized rapidly in the cytoplasm; preferentially concentrates histidine, arginine, serine, and glycine in accordance with the known composition of isolated KH; and is synthesized as the monomer with orderly polymerization to higher molecular weight species (Elias *et al.*, 1972).

The following information was sought: (1) The minimum concentration of l-histidine required for unimpeded protein synthesis to occur; (2) the reproducibility of the system; (3) the rate of incorporation of tritiated histidine as a function of time; (4) the time limits during which the system could be predictably used; and (5) the integrity of nucleic acid and protein biosynthetic pathways during the applicable time limits.

MATERIAL AND METHODS

Incorporation techniques

Dissection of epidermis from the posterior portion of bovine hoof was carried out as previously described (Ugel & Idler, 1970). Tissue was maintained in media lacking the precursor amino acid(s)

Address correspondence to: Peter M.Elias, M.D. Dermatology Section (111G), Veterans Administration Hospital, 4150 Clement Street, San Francisco, Calif. 94121, U.S.A.

to be studied at 4°C throughout the dissection. The basic medium was Medium 199 with or without 10–13% fetal calf serum (FCS), containing penicillin, 150 U/ml and streptomycin, 150 μg/ml, but lacking l-histidine when tritiated histidine was utilized as the pulse.

After dissection of epidermis to approximately 1 mm^3, the tissue was washed several times with cold media. The washed tissue was then divided into 3 or 5 g wet weight aliquots, depending on the experiment, and rapidly transferred to 125 ml Erlenmeyer flasks containing the appropriate, prewarmed media (10 ml/g tissue wet weight) and radioactive precursor. Incubations were carried out in a Fermentation Design (Model RSW61) shaker at 125 rev/min under a 5% CO_2/95% O_2 atmosphere at 37°C.

In these experiments l-histidine-2, 5-T (Amersham-Searle, Sp. Act. 50 Ci/nM) or l-histidine-3-T (New England Nuclear Corp., Sp. Act. 10 Ci/nM) were utilized. The viability of epidermal cells was checked at the 3rd and 21st h of incubation by pulsing for 3 hours with thymidine-methyl-T (New England Nuclear Corp., Sp. Act. 18 Ci/nM) followed by autoradiography as previously described (Elias *et al.*, 1972). Several samples pulsed with tritiated histidine were also assessed autoradiographically for integrity of protein synlthesis at time intervals ranging from 5 min to 24 h incubation.

Isolation of KH macro-aggregates

After the designated incubation times, the flasks were removed from the incubator, the supernatant culture fluid was decanted, and the tissue extracted with 1M potassium phosphate buffer, pH 7·0, for 15 min (10 ml/g) at 37°C. The phosphate extract was cleared twice for 30 min at 25,000 *g* at 4°C, and then dialyzed overnight at 0–4°C against 32 vol distilled H_2O. The turbid macroaggregates were then collected by centrifugation, washed, and solubilized in either 0·5 N sodium hydroxide or sodium decylsulphate (NaDS) (Ugel, 1971).

Scintillation counting

Small (100–500 μl) samples of the NaDS-solubilized macro-aggregates were mixed with 10 ml of Aquasol or a modification of Maickel's solution in glass scintillation vials (Elias *et al.*, 1972). Duplicate samples were counted in a Packard Tricarb Liquid Scintillation Spectrometer. The specific activity of the isolated material was calculated following determination of protein concentration (Lowry *et al.*, 1951).

Fractionation of isolated macro-aggregates

In these experiments, macro-aggregates were fractionated on 9% T/3% C polyacrylamide gels, using a multiphasic buffer system containing 0·02 M NaDS in the upper buffer (Ugel, Chrambach & Rodbard, 1971). 300–600 μl samples were fractionated until the glycinate-decylsulphate boundary approached the bottom of the gel. Staining of the fractionated homologous series with amido black, toluidine blue, and methyl-green pyronin was performed as previously detailed (Ugel, 1971; Ugel & Idler, 1972).

RESULTS

In vitro *system*

The system is useful for incubations of up to 24 h when tissue death is heralded by a progressive acid shift in media pH. The viability of epidermal basal and spinous cells was demonstrated by the incorporation of tritiated thymidine and uridine into large numbers of cells after 6–24 h *in vitro* (Fig. 1). Furthermore, the progressive accumulation of labelled precursors within KH granules evident up to 24 h (Fig. 2) suggests that *de novo* protein synthesis continued to occur throughout the incubation (see below). Numerous labels are also found in regions of the cytoplasm where KH granules are

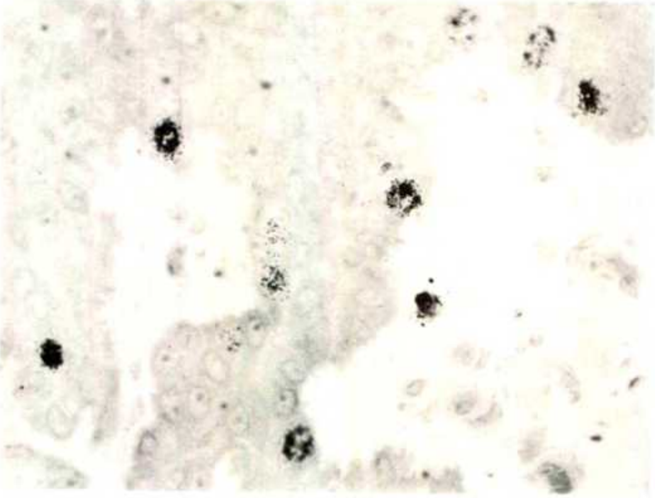


FIGURE 1. Several basal cells incorporate ^{3}H-thymidine into their nuclei. Total incubation, 6 h; thymidine pulse, 3 h. Similar incorporation occurred between 21 and 24 h (× 320).

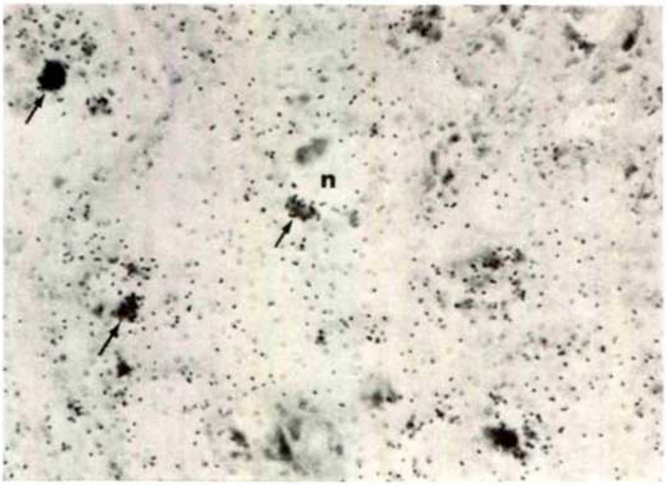


FIGURE 2. Granular cells demonstrate heavy ^{3}H-histidine concentration over several large KHG (arrows); scattered cytoplasmic labelling often appears to be associated with small KH granules, while nuclei (N) are consistently spared. Total incubation and histidine pulse, 24 h (× 300).

not seen. These may be associated with granules beneath the resolution of light microscopy, or within nascent, newly synthesized KH monomers (Elias *et al.*, 1972).

Keratohyalin granules generally, and KH fractionated on polyacrylamide gels (Fig. 3), remained intact and stained intensely with acid dyes, although some diminution in histochemical stains for RNA (toluidine blue, pyronin Y) was noted beyond 6 h *in vitro* in comparison to controls.

Reproducibility (*Table* 1)

Three separate experiments, each utilizing six to eight parallel flasks with a standard 6-h pulse, and one experiment of five flasks and a 4-h pulse, were performed. Since the amount of radioactivity and time in each experiment were constant, the observed differences in specific activities from experiment to experiment could reflect: (a) variations in the final minced size of the tissue; (b) age and/or sex of animals; (c) time between slaughter and onset of incubations, etc.

As seen in Table 1, the maximum standard deviation in specific activity in any of the four experiments was ±23,000 while the average deviation from the mean was 11·9%. However, the wide variations in specific activities from experiment to experiment indicate that data must be internally compared, and that results from several different experiments cannot be pooled.

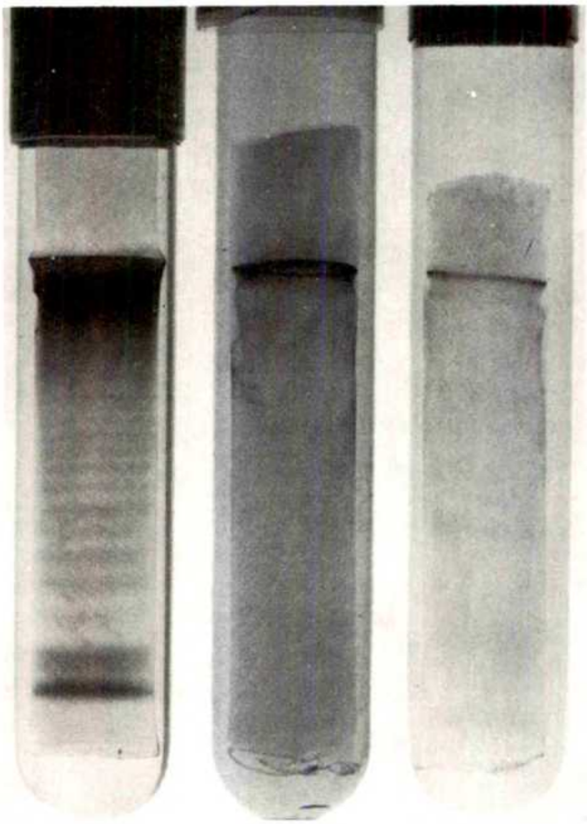


FIGURE 3. Fractionated keratohyalin oligomers, isolated after 6 h incubation. Homologous series is stained by amido black (left), but not by toluidine blue (centre) or methyl-green pyronin (right). Oligomers isolated and fractionated prior to incubations are stained by both toluidine blue and methyl-green pyronin (see Ugel, 1971).

Kinetics of precursor incorporation

Labelling of KH *in vitro* with amino acid precursors proceeds more rapidly when media lacking the same cold amino acid is utilized in place of media containing additional cold amino acids. However, with currently available, high specific activity tritiated precursors, the minute amino acid concentration provided by the labelled amino acid alone can be rate limiting. We found that unimpeded incorporation occurred with media l-histidine concentrations as low as 0·05 μg/ml, levels that were exceeded several-fold in these experiments (Elias, P.M. & Montague, P., unpublished observations).

In vitro incorporation of tritiated l-histidine into bovine keratohyalin assumed the following pattern (Fig. 4a): Incorporation began rather slowly between 0 and 2 h, accelerating between 2 and 6 h. However, incubation in FCS-free media resulted in further acceleration of protein synthesis between 2 and 6 h (Fig. 4a & b). Experiments were not done to differentiate whether serum generally is inhibitory, or if free l-histidine present in FCS decreases the rate of incorporation by competitive inhibition. When tissue was pre-incubated for 6 h in histidine-free, FCS-free media, and then pulsed, more rapid incorporation occurred between 0 and 4 h (Fig. 4a).

TABLE 1. Reproducibility after standard incubations

Exper.	Time (h)	Flask	Spec. act. (cpm/mg prot.)	% Dev.
A	6	1	284,250 (±22,718 = 1 s.d.*)	+ 8·8
		2	233,466	−10·7
		3	271,568	+ 4·2
		4	284,289	+ 8·8
		5	236,825	− 9·2
		6	254,286	− 2·7
			x = 260,785	
B	6	1	109,514 (±14,196 = 1 s.d.)	− 4·3
		2	115,573	+ 0·9
		3	110,983	− 3·5
		4	142,876	+24·3
		5	95,854	−17·4
		6	118,825	+ 3·5
		7	112,826	− 1·7
			x = 115,207	
C	6	1	112,240 (±13,917 = 1 s.d.)	+ 6·6
		2	111,100	+ 5·7
		3	94,280	−10·4
		4	91,195	−13·3
		5	129,207	+22·8
		6	86,727	−17·1
		7	102,783	− 1·9
		8	111,022	+ 5·7
			x = 104,819	
D	4	1	53,175 (±6,045 = 1 s.d.)	+18·0
		2	49,440	+ 9·0
		3	39,545	−12·0
		4	39,806	−11·0
		5	43,286	− 4·4
			x = 45,050	

* Standard deviation.

DISCUSSION

Epidermal protein synthesis has been studied in several *in vivo* and *in vitro* systems (reviewed in Bernstein *et al.*, 1970; Freedberg, 1972). A histidine-rich moiety which resembles the composition of bovine keratohyalin has been isolated from rat epidermis by Sibrack, Gray & Bernstein (1974). Other biochemically differing keratohyalin fractions have been isolated by Tezuka & Freedberg (1972) and

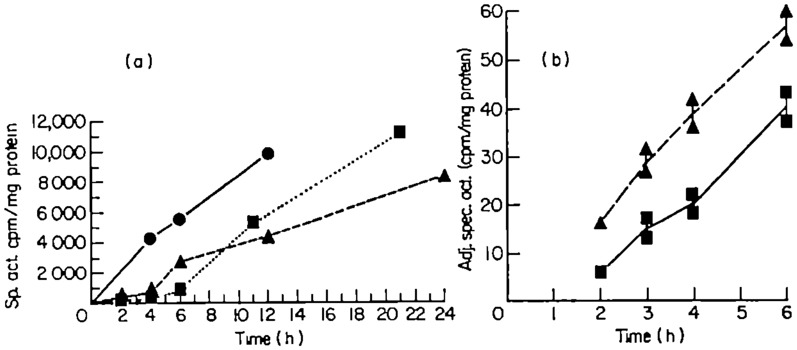


FIGURE 4. (a) Experiment in which effects of: (1) incubation without FCS and pre-incubation for 6 h prior to pulsing were compared to usual incorporation pattern observed with FCS and pulse added at beginning of incubation (see text). ■- - -■, with FCS; ▲- - -▲, without FCS; ●——●, without FCS after 6 h pre-incubation.

(b) Effect of eliminating FCS on early incorporation is illustrated in more detail. Brackets represent range of specific activities (× 10^2) of two flasks assayed at each time period. ■——■, with FCS; ▲- - -▲, without FCS.

Matoltsy, Lavker & Matoltsy (1974). The diversity of the results obtained suggests that keratohyalin granules *in vivo* are probably heterogeneous in structure and perhaps in function (Brody, 1959; Fukuyama & Epstein, 1969; Lavker and Matoltsy, 1970). In our opinion, the most useful feature of bovine keratohyalin lies in its *uniformity*. Thus, modulations of precursor incorporation into bovine KH in response to pharmacological agents can be reliably monitored (see applications below). Data which have already accrued from this *in vitro* system (Elias *et al.*, 1972) include: (1) correlative autoradiographic and biochemical studies demonstrating concentration of arginine, serine, histidine, and glycine preferentially into *in situ* KH granules and into fractionated KH macro-aggregates; (2) demonstration by autoradiography of incorporation of amino acid precursors into KH granules as early as 5 min after pulsing, indicating that KH is probably synthesized in the cytoplasm, presumably by ribosomes which surround granules; (3) absence of nuclear labelling by histidine within granular cells at all time periods from 5 min to 24 h, indicating that the histidine-rich portion of KH is probably not of nuclear origin; and, (4) demonstration that KH is synthesized initially as the monomer, with progressive polymerization to higher molecular weight oligomers occurring subsequently as a function of time.

The experiments described here delineate several further characteristics of the *in vitro* bovine epidermal system. Morphologically, granules remain grossly intact and stain intensely with acid dyes up to 24 h *in vitro*, but the RNA staining qualities of *in situ* and fractionated bovine KH are visibly altered after 6 h *in vitro*. Loss of RNA stainability from granules could reflect extraction of the entire keratohyalin RNA-protein complex, detectable earlier by a loss of RNA stainability than by protein staining, or it might be due to *in vitro* activation of endogenous ribonuclease resulting in a more specific attack on the RNA moiety. It would be of interest to see whether addition of RNAase inhibitors would restore a normal staining pattern.

Unrestricted incorporation of tritiated histidine into KH occurred even with concentrations of l-histidine as low as 0·05 μg/ml. Similar data need to be derived for other amino acids before kinetic experiments can be confidently performed utilizing other tracers in media lacking the particular amino acid being used as the pulse. Under the conditions discussed above, incorporation of tritiated histidine into KH appeared to increase linearly between 4 and 24 h as a function of time. The lower rate of incorporation with FCS before 4 h may have been due to competitive inhibition from an intracellular pool of l-histidine which required 2–4 h to be depleted, since linear kinetics occurred thereafter, and furthermore, pre-incubation in histidine-free, FCS-free media resulted in rapid early incorporation, probably due to depletion of intracellular cold histidine. However, it could be argued that several hours are required for the protein synthetic apparatus to 'recover' from the shock of handling prior to incubation. The slow rise probably cannot be attributed to competitive inhibition by l-histidine derived from conversion of other amino acids to l-histidine, since such interconversion pathways have not been described in mammals.

Potential applications of the in vitro *bovine epidermal system*

Either hyper- or hypogranulosis is associated with several important skin diseases, notably psoriasis and ichthyosis. Since incorporation into KH occurs in a reproducible and linear fashion, this system provides unique opportunities to evaluate drugs of potential interest to clinical dermatology. Drug effects in the *in vitro* system could be correlated with clinical and histological response in patients, and multiple agents could be screened prior to clinical trials. We have already described several agents which markedly diminish protein synthesis (Elias *et al.*, 1972), and studies are underway in this laboratory to assay the effects of cyclic AMP analogues, retinoic acid, and steroids on KH synthesis as well.

ACKNOWLEDGMENTS

We are indebted to Dr Ervin Epstein, Jr. and Dr Robert Briggaman for their critical appraisal of the manuscript.

REFERENCES

BERNSTEIN, I.A., CHAKRABATI, S.G., KUMAROO, K.K. & SIBRACK, L.A. (1970) Synthesis of protein by mammalian epidermis. *Journal of Investigative Dermatology*, **55**, 291.

BRODY, I. (1959) The keratinization of epidermal cells of normal guinea-pig skin as revealed by electron microscopy. *Journal of Ultrastructure Research*, **2**, 482.

ELIAS, P.M., MONTAGUE, P.M. & UGEL, A.R. (1972) *In vitro* studies on the kinetics, composition, and homology of bovine keratohyalin. *Experimental Cell Research*, **73**, 95.

FREEDBERG, I.M. (1972) Pathways and control of epidermal protein synthesis. *Journal of Investigative Dermatology*, **59**, 56.

FUKUYAMA, K. & EPSTEIN, W.L. (1969) Sulfur-containing proteins and epidermal keratinization. *Journal of Cell Biology*, **40**, 830.

GUSS, S.B. & UGEL, A.R. (1972) Immunofluorescent antibodies to bovine keratohyalin and immunologic confirmation of homology. *Journal of Histochemistry and Cytochemistry*, **20**, 97.

LAYKER, R.M. & MATOLTSY, A.G. (1970) Formation of horny cells. The fate of cell organelles and differentiation products in ruminal epithelium. *Journal of Cell Biology*, **44**, 501.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reaction. *Journal of Biological Chemistry*, **193**, 265 and 275.

MATOLTSY, A.G., LAYKER, R.M. & MATOLTSY, M.N. (1974) Demonstration of cystine-containing protein in keratohyalin granules of the epidermis. *Journal of Investigative Dermatology*, **62**, 406.

SIBRACK, L.A., GRAY, R.H. & BERNSTEIN, I.A. (1974) Localization of the histidine-rich protein in keratohyalin: A morphologic and macromolecular marker in epidermal differentiation. *Journal of Investigative Dermatology*, **62**, 394.

TEZUKA, T. & FREEDBERG, I.M. (1972) Epidermal structural proteins. I. Isolation and purification of keratohyalin granules of the newborn rat. *Biochemical Biophysical Acta*, **261**, 402.

UGEL, A.R. & IDLER, W. (1970) Stratum granulosum: Dissection from cattle hoof epidermis. *Journal of Investigative Dermatology*, **55**, 350.

British Journal of Dermatology (1976) 95, 123.

A study of the permeability barrier in epidermis and oral epithelium using horseradish peroxidase as a tracer *in vitro*

C.A.SQUIER* AND ROSAMUND M.HOPPS

Department of Oral Pathology, The London Hospital Medical College, Turner Street London, E.1.

Accepted for publication 8 December 1975

SUMMARY

Small biopsies of skin, and of keratinized and non-keratinized oral mucosa were incubated with horseradish peroxidase, fixed and reacted to demonstrate peroxidase activity at the light and electron microscope level. In all tissues peroxidase extended through the intercellular spaces of the epithelium up to, but not into, the stratum corneum of keratinized, or the superficial layer of non-keratinized, tissue. This distribution corresponds to that seen in studies using the tracer *in vivo* where the presence of an intercellular permeability barrier has been demonstrated in the upper cell layers. It is suggested that the method is a useful way of investigating the permeability of normal and pathological human epithelial tissues.

One of the important properties of the mammalian epidermis is that of a barrier which limits the loss of water from the body surface while at the same time restricting the entry of material from the exterior; this latter function is also shared by the epithelium lining the oral cavity (Kaaber, 1974; Squier & Johnson, 1975). The use of microscopically detectable tracer substances provides a useful method for visualizing the pathways that certain substances take in crossing the epidermis or oral epithelium and for revealing the presence of any barrier to penetration. Tracers that have been employed in this way include ferritin (Nordquist, Olson & Everett, 1966), lanthanum (Wolff & Schreiner, 1968; Komura, Shimada & Ofuji, 1969), ruthenium red (Schreiner & Wolff, 1971), Thorotrast (Wolff & Hönigsmann, 1971) and horseradish peroxidase (Schreiner & Wolff, 1969; McDougall, 1970, 1971; Squier, 1973; Elias & Friend, 1975).

Most of these permeability studies have been carried out on experimental animals in which horseradish peroxidase, an enzyme protein of molecular weight approximately 40,000, has been injected intravenously or sub-epithelially. Since the injection or topical application of tracer substances to human subjects raises ethical problems, the use of biopsies would seem a useful approach for studying permeability. This has been tried in the past by immersing excised skin in fixative solutions containing lanthanum (Hashimoto, 1971) but such a procedure obviously cannot give a very realistic indication of the permeability of vital tissue. We have therefore attempted to use biopsies of fresh tissue for studying the permeability of the epidermis and oral mucosa.

---

* Present address: Dental Research, College of Dentistry, University of Iowa, Iowa City, Iowa 52242, U.S.A.

MATERIAL AND METHODS

Biopsies were taken from the keratinized oral mucosa of the rat, rabbit and monkey palate (8 biopsies), from the glabrous chest skin of the macaque monkey (3 biopsies) and from the forearm (flexor surface), thigh and back of human subjects (11 biopsies). Non-keratinized epithelium was obtained from rabbit buccal mucosa (7 biopsies), which closely resembles human buccal mucosa (Chen, 1970), and from monkey lip mucosa (3 biopsies). For comparative purposes 2 specimens each of rabbit cornea, liver and intestine were also taken. A total of 38 biopsies were studied.

Specimens were removed under general anaesthesia except from the human subjects where half of the biopsies were taken after infiltration of the adjacent skin with 1% lignocaine and the remainder without anaesthetic by using a high speed rotating punch. All biopsies were subdivided into blocks not exceeding 2 mm in any one dimension and immediately incubated at 37°C in horseradish peroxidase (Sigma type II) freshly prepared as a 3% solution in normal saline. After 1 h the tissue blocks were fixed, without rinsing, for 20 min either in buffered formaldehyde solution for light microscopy, or in buffered 2% formaldehyde–2·5% glutaraldehyde (Karnovsky, 1965) for electron microscopy. They were then washed in 3 changes of 0·1 M cacodylate buffer over 45 min.

Tissue destined for light microscopy was orientated on cork disks using Tissue-Tek and a coverslip for support as described by Mackenzie (1975). The corks were quenched in isopentane cooled in liquid nitrogen and the specimens stored in liquid nitrogen until sectioning was convenient (this never exceeded 3 months).

Sections of the frozen blocks were cut at 5 μm in a cryostat at -15°C and collected on coverslips coated with a glycerine–albumin mixture to facilitate adhesion. Orientation was checked using toluidine-blue stained sections to obtain sections most nearly perpendicular to the epithelial surface. The coverslips were placed in Columbia jars, washed for 7–10 min in two changes of 0·1 M tris-maleate buffer and incubated for 10 min in a medium containing hydrogen peroxide and diaminobenzidine (Graham & Karnovsky, 1966). After rinsing in distilled water the specimens were mounted in glycerine jelly for examination with the light microscope.

For electron microscopy, fixed blocks of skin and keratinized oral mucosa were embedded in molten 7% agar at 40°C and 35–40 μm slices prepared on a Sorvall tissue chopper. The slices were then rinsed in 0·1 M tris-maleate buffer, pre-incubated in a tris-maleate buffered solution of diaminobenzidine for 30 min in order to aid diffusion through the relatively large blocks, and then incubated

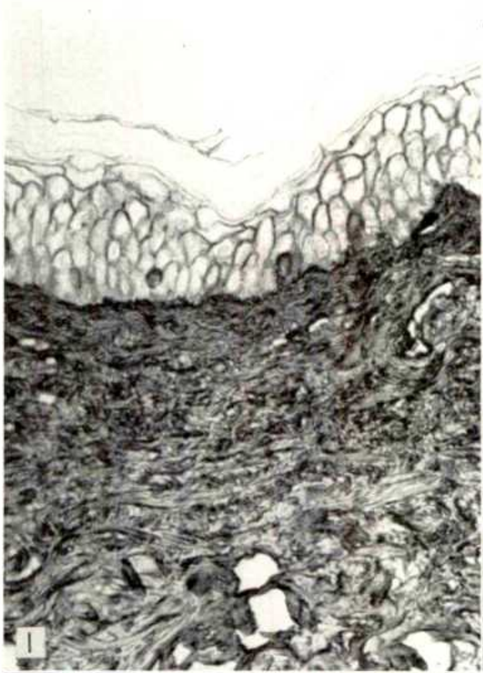
---

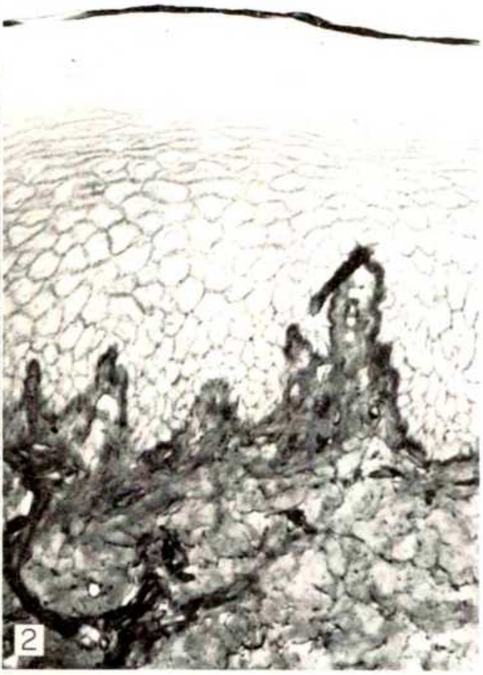
FIGURE 1. Section through human skin after incubation in horseradish peroxidase. The dark reaction product is present throughout the connective tissue and extends through the intercellular spaces of the epidermis as far as the stratum corneum. A light deposit is present on the surface of the epidermis (×375).

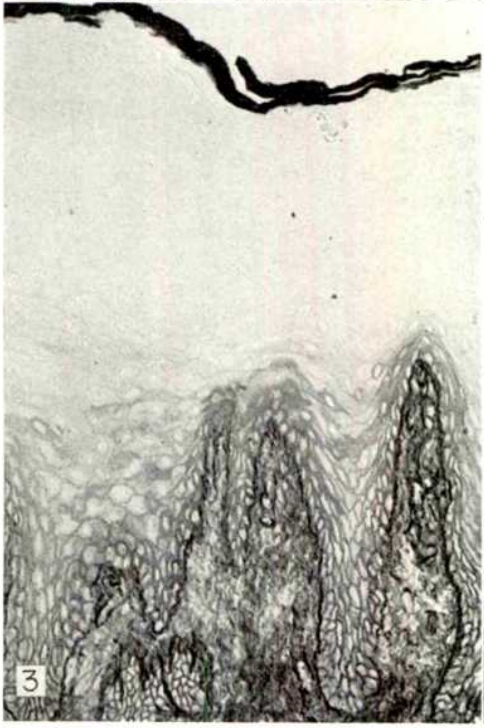
FIGURE 2. A section through keratinized palatal mucosa from monkey, treated as in Fig. 1. The reaction product again stops short of the keratinized layer and although a heavy deposit is seen over the surface of the epithelium, no peroxidase has entered the surface layer (×300).

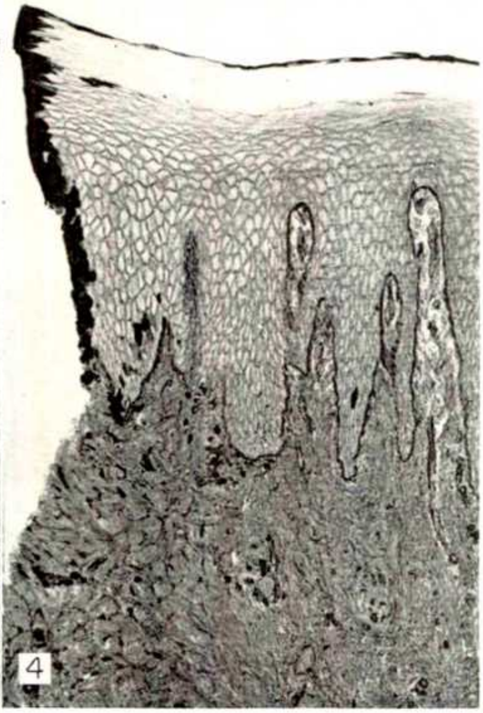
FIGURE 3. Non-keratinized oral mucosa from rabbit cheek in which the tracer has penetrated approximately one third of the way through the epithelium. The epithelium is considerably thicker than in keratinized tissue (compare with Figs 1 & 2). There is a very heavy surface deposit that appears to have entered the desquamating superficial cell layers (×215).

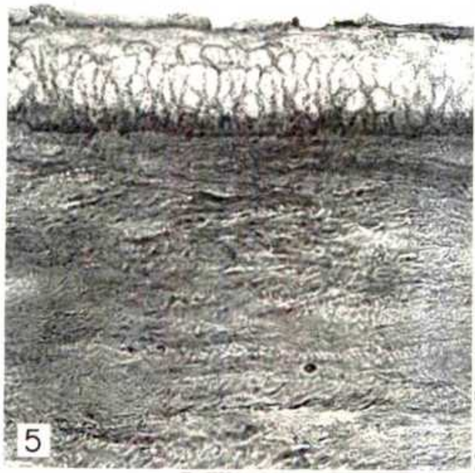
FIGURE 4. The edge of a section through keratinized oral mucosa from the rabbit palate. There has been slight lateral penetration of tracer from the cut edge of the block but the barrier layer in the stratum corneum is still conspicuous (×130).


1


2


3


4


5

6

7

for a further 15 min in the complete incubating medium, as above. After rinsing in distilled water, the tissue was osmicated in a 1% OsO_4 solution for $1\frac{1}{2}$ h, dehydrated and embedded in Epon. Ultrathin sections were examined unstained or stained with lead citrate only in an AEI EM6B electron microscope.

Controls for endogenous peroxidase

Blocks of oral mucosa that had not been incubated with horseradish peroxidase were prepared for light microscope examination, as above.

RESULTS

Light microscopy

All histological sections of skin and keratinized oral mucosa showed a strong reaction for horseradish peroxidase in the connective tissue and this extended through the intercellular spaces of the epithelium as far as the stratum corneum (Figs 1, 2, 4). The surface of the epithelium was frequently covered with a thin layer of reaction product but this never entered the intercellular spaces of the stratum corneum, which therefore appeared as a clear unstained layer in all preparations. There appeared to be no difference in the penetration of horseradish peroxidase into the skin biopsies taken with or without local anaesthesia.

In non-keratinized oral epithelium, the peroxidase was again seen in the connective tissue and around the cells of the deeper one-third of the epithelium (Fig. 3). The remaining superficial portion was unstained although a reaction could usually be seen over the surface of the epithelium. Non-keratinized oral epithelium is generally thicker than keratinized oral epithelium and epidermis, (compare Figs 1, 2 and 3) and the clear zone appeared correspondingly wider.

Most sections of epithelium, from whatever tissue examined, showed an 'edge-effect' where the peroxidase had entered all layers of the epithelium laterally for a distance of approximately 100 μm (Fig. 4). This is almost certainly a consequence of surface damage arising during the dividing of the tissue blocks. There was no appreciable difference in the distribution of reaction product in sections taken at levels throughout the blocks.

The epithelium of both cornea (Fig. 5) and intestine appeared to have been entirely penetrated by the peroxidase, as were the blocks of liver tissue.

Sections from tissue that had not been treated with horseradish peroxidase showed slight reaction for endogenous peroxidase in the connective tissue (Fig. 6). This was principally localized over erythrocytes and polymorphonuclear leukocytes. The epithelium did not react.

Electron microscopy

Examination of keratinized tissue with the electron microscope confirmed that the peroxidase had entered the intercellular spaces of the epithelium and penetrated as far as the junction between the granular and keratinized layers (Fig. 7). Here the reaction product stopped abruptly and was not seen

FIGURE 5. A section of rabbit corneal epithelium through which the tracer appears to have penetrated completely (× 450).

FIGURE 6. A control section of rabbit cheek mucosa showing endogenous peroxidase activity. This is located principally over cells in the connective tissue, probably erythrocytes and leukocytes, while the epithelium is free from reaction (× 112).

FIGURE 7. An electron micrograph of rat palatal epithelium showing intercellular penetration of the horseradish peroxidase as far as the keratinized layer (K). The limit of penetration is well defined (× 5000).

between the succeeding squames of the stratum corneum. Peroxidase was also evident over the surface squames of the tissue where it occasionally penetrated between the outermost one or two layers, but never further.

DISCUSSION

The presence of a barrier in the superficial part of the epithelium could be clearly demonstrated in both keratinized and non-keratinized tissue. On the other hand peroxidase passed through corneal and intestinal epithelium, both thought to lack any permeability barrier (Mishima & Hedbys, 1967; Cornell, Walker & Isselbacher, 1971), and penetrated blocks of liver of similar volume to those of the skin and the mucosa. This suggested that the barrier limiting peroxidase in tissue blocks *in vitro* is real and not just an artifact due to the distances the tracer has to diffuse through the tissue; a conclusion confirmed by cutting sections through the full thickness of a block when no variation in the distribution of the reaction product was encountered. The results obtained by incubating blocks of skin and mucosa in horseradish peroxidase are identical to those of workers who have administered this tracer by local or systemic injection into living animals and human subjects.

A number of ultrastructural studies have suggested that the epithelial permeability barrier is produced by the 'membrane coating granules' (or Odland bodies) which are extruded into the intercellular space in the superficial part of the granular layer (Schreiner & Wolff, 1969; Squier, 1973; Lavker, 1974; Elias & Friend, 1975). Since topically applied tracer fails to penetrate more than 1–3 cell layers into the surface of the epithelium, whether keratinized or not, it would seem that the barrier produced by the membrane coating granules persists throughout the stratum corneum. This is confirmed by results from the present work, for although there was the opportunity for tracer to enter the intercellular spaces of the stratum corneum laterally, through the cut edges of the tissue blocks, there was no significant penetration into the layer from this direction, apart from the slight 'edge effect' already mentioned. Similar results have been reported after blocks of epidermis were treated with lanthanum salts in fixative solution and examined in the electron microscope (Hashimoto, 1971).

While it is often unwise to argue from the *in vitro* to the *in vivo* situation, it is clear from our findings that the epithelium behaves in a similar way when incubated with horseradish peroxidase as when the tracer is actually applied to, or injected into, the intact tissue. This makes the *in vitro* method a useful means of examining permeability in human subjects without the need to bring tracer substances into contact with the patient; similar approaches have been of great value in studying epithelial proliferation using radio-isotopes (Lachapelle, 1969).

The small size of biopsy required and the rapidity with which cryostat sections can be prepared and reacted also make it a valuable method for investigating the permeability of lesional areas, and we have successfully used this approach to look at certain hyperkeratotic skin lesions (Squier & Eadie; unpublished results).

ACKNOWLEDGMENTS

We acknowledge the assistance of Dr Robin Eadie, St John's Hospital for Diseases of the Skin, London, W.C.1 in obtaining biopsies of human skin. The work was supported by an M.R.C. grant for technical assistance.

REFERENCES

CHEN, S.-Y. (1970) Comparison of the fine structure of cheek and hard palate in the rabbit. *M.S. Thesis*, University of Illinois at the Medical Center, Chicago, Illinois.

CORNELL, R., WALKER, W.A. & ISSELBACHER, K.J. (1971) Small intestinal absorption of horseradish peroxidase. *Laboratory Investigation,* **25**, 42.

ELIAS, P.M. & FRIEND, D.S. (1975) The permeability barrier in mammalian epidermis. *Journal of Cell Biology,* **65**, 180.

GRAHAM, R.C. & KARNOVSKY, M.J. (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney; ultrastructural cytochemistry by a new technique. *Journal of Histochemistry and Cytochemistry,* **14**, 291.

HASHIMOTO, K. (1971) Intercellular spaces of the human epidermis as demonstrated with lanthanum. *Journal of Investigative Dermatology,* **57**, 17.

KAABER, S. (1974) The permeability and barrier functions of the oral mucosa with respect to water and electrolytes. *Acta odontologica Scandinavica,* **32**, Supplement 66.

KARNOVSKY, M.J. (1965) A formaldehyde–glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology,* **27**, 137A.

KOMURA, J., SHIMADA, Y. & OFUJI, S. (1969) Lanthanum staining of the epidermal intercellular spaces. An electron microscopic study. *Archiv für klinische und experimentelle Dermatologie,* **235**, 221.

LACHAPELLE, J.M. (1969) Isotopic labelling of cutaneous structures. *British Journal of Dermatology,* **81**, 229.

LAVKER, R. (1974) The fate of membrane coating granules in oral epithelium. *Journal of Cell Biology,* **63**, 186a.

MACKENZIE, I.C. (1975) A simple method of orientation and storage of specimens for cryomicrotomy. *Journal of Periodontal Research,* **10**, 49.

MCDOUGALL, W.A. (1970) Pathways of penetration and effects of horseradish peroxidase in rat molar gingiva. *Archives of Oral Biology,* **15**, 621.

MCDOUGALL, W.A. (1971) Penetration pathways of a topically applied foreign protein into rat gingiva. *Journal of Periodontal Research,* **6**, 89.

MISHIMA, S. & HEDBYS, B.O. (1967) The permeability of the corneal epithelium and endothelium to water. *Experimental Eye Research,* **6**, 10.

NORDQUIST, R.E., OLSON, R.L. & EVERETT, M.S. (1966) The transport, uptake, and storage of ferritin in human epidermis. *Archives of Dermatology,* **94**, 282.

SCHREINER, E. & WOLFF, K. (1969) Die Permeabilität des epidermalen Intercellularraumes für kleinmolekulares Protein. *Archiv für klinische und experimentelle Dermatologie,* **235**, 78.

SCHREINER, E. & WOLFF, K. (1971) Der interzellularraum der Epidermis. *Acta Histochemica,* Supplement **10**, 173.

SQUIER, C.A. (1973) The permeability of keratinized and nonkeratinized oral epithelium to horseradish peroxidase. *Journal of Ultrastructure Research,* **43**, 160.

SQUIER, C.A. & JOHNSON, N.W. (1975) The permeability of oral mucosa. *British Medical Bulletin,* **31**, 169.

WOLFF, K. & SCHREINER, E. (1968) An electron microscopic study on the extraneous coat of keratinocytes and the intercellular space of the epidermis. *Journal of Investigative Dermatology,* **51**, 418.

WOLFF, K. & HÖNIGSMANN, H. (1971) Permeability of the epidermis and the phagocytic activity of keratinocytes—ultrastructural studies with Thorotrast as a marker. *Journal of Ultrastructure Research,* **36**, 176.

British Journal of Dermatology (1976) 95, 131.

Study of an inhibiting factor of epidermal proliferation in plucked skin and various tumours of mice

M.GUIGON, E.FRINDEL, J.HEWITT AND M.TUBIANA

Institut de Radiobiologie Clinique, Inserm U66, Villejuif, and Laboratoire de Recherches Dermatologiques, Hôpital Tarnier, Paris, France

Accepted for publication 24 November 1975

SUMMARY

The aim of our research was to investigate, by autoradiographic methods, the presence of an inhibitory factor (IF) in normal skin, in plucked skin and in five experimental skin tumours of the mouse, capable of inhibiting epidermal proliferation. The experimental model used was that of Bullough & Laurence (1960), i.e. a wound in the ear of a mouse. After tritiated thymidine administration the measurement of the labelling index confirmed the existence of an inhibitory substance in normal skin. The plucked skin had no inhibitory effect when it was taken 24 h after plucking, but after 4 days its effect had returned to normal. The skin tumours, whether epithelial or mesenchymal, showed the same inhibitory effect as normal skin whereas a non-cutaneous tumour tested had no inhibitory effect.

In 1960, Bullough & Laurence, working on the epidermis of the ear in mice, suggested the existence of a diffusible substance capable of specifically inhibiting cellular division in the epidermis. Later, numerous studies of this substance, named chalone by Bullough (1962), were made in the skin (Marks, 1972; Iversen, Bhangoo & Hansen, 1974) and in other normal tissues or tumours (Houck & Hennings, 1973; Bullough, 1973).

Our work has been centered on the study of plucked skin and cutaneous tumours, in which cellular division of the epidermis is above normal. We were interested to know whether these actively dividing populations retain the capacity to inhibit proliferation of the skin. As the 'chalones' seem to act locally by diffusion, the mouse ear, as shown by Bullough & Laurence (1960), seems to be an adequate model for such studies. The ear comprises two cutaneous sheets less than 1 mm apart separated by a piece of cartilage. We chose whole tissue fragments as a source of inhibitory factor and have tested their effect on epidermal proliferation *in vivo* by autoradiography as did Finegold (1965) and Yamaguchi *et al.* (1974). This method permits the determination of the percentage of cells in DNA synthesis, which can be grossly considered as an indicator of proliferation. In a preliminary study (Guigon *et al.*, 1973),

Correspondence address: Dr M.Guigon, Institut de Radiobiologie Clinique 16 Bis, avenue Paul-Vaillant Couturier, 94800 Villejuif, France.

we have been able, by this technique, to confirm the inhibitory effect of the skin of the mouse and the tissue specificity.

MATERIALS AND METHODS

Mice

Fifty-nine male C_3H mice were used as donors and recipients; in each group, there were at least three recipients (one ear per mouse) with the exception of the last two groups which comprised only two mice each.

Wounds

After anaesthetising the mice with Nembutal (75 mg/kg intraperitoneally), a cutaneous fragment of approximately 0·5 cm^2 was removed from the exterior aspect of one ear, using a small scalpel blade so as not to damage the cartilage. A fragment of normal or tumourous tissue was applied in such a way as to overlap the lesion completely. The skin fragments were taken from the backs of mice killed immediately before application to the ear lesion of the recipient mouse. The fragment was maintained in place for 4 h by a plastic cast covering the ear. All experiments were initiated between 10 and 11 o'clock a.m. to avoid circadian variations. Twenty hours after the fragment was removed, the mice were injected intraperitoneally with tritiated thymidine (50 μCi/0·5 ml). 30 min after the injection, the mice were killed, the ears removed, fixed (Carnoy for 1 h, then 10% formaldehyde) and mounted in paraffin wax. The number of recipient mice used in each experiment is indicated in Table 1; the following series were studied:

Controls. Wound alone; normal skin of the dorsal region applied to the wound.

Plucked skin fragments. The dorsal region was plucked by hand either 24 h or 4 days before the experiment. In order to estimate the labelling index of plucked skin, three mice were injected with tritiated thymidine 24 h and 4 days after plucking (50 μCi/0·5 ml intraperitoneally).

TABLE 1. Labelling index of the epidermis situated opposite a wound or wound having had a fragment of normal or tumourous tissue applied to it

	No. of recipients	Labelling index (%) mean ± s.d.	% Inhibition
Normal epidermis of the ear	7	2 ± 1	—
Wound	10	22·8 ± 3·7	—
Wound + skin			
Normal skin	11	12·5 ± 2·1*	45
Plucked skin			
24 h after plucking	8	25·7 ± 7·2 n.s.	0
4 days after plucking	4	8·9 ± 2·5*	61
Wound + tumour			
Epidermoid carcinomas	9	13·5 ± 4·7*	40
Mammary tumour	3	12·3 ± 1·62*	46
NCTC fibrosarcoma	3	8·5 ± 1·7*	63
EMT6 tumour	2	10·8	53
Thymic lymphoma	2	22·3	0

* Labelling index differs significantly from that of the 'wound' group ($P < 0·001$); n.s. = not significant ($P > 0·05$).

Tumourous fragments. They were either cutaneous tumours: epidermoid carcinoma of keratinizing type, mammary tumour (Le François *et al.*, 1971), the NCTC 2472 fibrosarcoma (Sanford, Likely & Earle, 1954) and the EMT6 tumour (Rockwell, Kallman & Fajardo, 1972) or a non cutaneous tumour, i.e. the thymic lymphoma of the AKR mice (Furth, Siebold & Rathbone, 1933).

Autoradiography

Histological sections of about 4 μm were taken at right angles to the cartilage. A preliminary histological examination was carried out in order to exclude those lesions in which the cartilage had been ruptured. The sections at 1 mm from the edges of the wound were selected for the studies since they corresponded to about the centre of the wound. Autoradiography was carried out by dipping in Ilford K2 nuclear emulsion, the slides being exposed for 3 weeks at 4°C, developed and stained with haematoxylin–eosin.

The labelling index (LI) of the nucleated interfollicular epidermal cells was evaluated in the normal epidermal portion opposite to the wound. The mean background was about 0·8 grains per nucleus. All cells whose nuclei contained three grains or more under the objective × 100 and the eyepieces × 12·5 were considered as labelled. The length of the epidermis examined was determined by the length of the wound; since only a small number of cells (421 ± 165) per specimen were available, several sections cut from the same fragment were counted and an average labelling index was established. The LI was established on all cells and not on basal cells only. The statistics were calculated as standard deviations (s.d. of the means of the number of animals and not on the means of the number of histological sections). The s.d. was not calculated in cases where there were less than 3 animals per point.

RESULTS

Table 1 shows the average labelling indices of normal epidermis situated opposite to a wound alone or to a wound which had had a tissue fragment in contact with it for four hours. Control values are included in the table. Statistical analysis was carried out by the Student's '*t*'-test for small samples.

The average labelling index of the normal epidermis measured along the whole length of one side was about 2%. Twenty-four hours after wounding, the labelling index in the normal epithelium facing the wound was markedly increased (22·8%).

When a fragment of normal skin was in contact with the wound during the 4 h, the labelling index of the epidermis decreased to 12·5%. The difference between these two values is very significant ($P < 0·001$).

When plucked skin, instead of normal skin, was in contact with the wound, the results differed depending on the time interval between the plucking and the experiment:

Skin removed 24 h after plucking had no inhibitory effect on the proliferation of normal epidermis, the labelling index of the epidermis facing the wound being 25·7%; this value is slightly higher than that found after wounding alone, but the difference is not statistically significant.

With regard to skin taken 4 days after plucking, the labelling index was 8·9%; this result shows a marked (61%) and significant ($P < 0·001$) inhibitory action. The inhibition under these conditions is greater than that seen after contact with normal skin and the difference between these two types of skin is significant ($P < 0·02$).

It should be noted that the labelling index of the plucked skin was 23·4%, 24 h after plucking and 4·02% 4 days after plucking.

After contact with a fragment of tumourous tissue, the labelling index of the epidermis was not decreased with thymic lymphoma (22·3%) when compared to that which was seen in the controls, i.e. wound alone with no fragment present. The observed values for the other tumours were significantly lower than those seen in the controls: 13·5% with epitheliomas, 12·3% with mammary tumour, 10·8% with EMT6 tumour and 8·5% with NCTC fibrosarcoma. These results indicate an inhibitory effect on epithelial proliferation ($P<0{\cdot}001$) which is not significantly different from that observed when normal skin is in contact with the wound, except in the case of the NCTC 2472 fibrosarcoma.

DISCUSSION

The observations we made using whole tissue fragments as a source of inhibiting factor confirm the results obtained by others (Bullough & Laurence, 1964; Elgjo, 1969) with aqueous extracts. The advantage of this technique is that one is able to use the tissues without any preparation and only a small fragment is necessary to study the presence or absence of an inhibitor.

The data we present may underestimate the phenomena we describe since the duration of contact between the fragments and the wound and the time interval between the removal of the fragment and the injection of the thymidine may not be optimal. However, even though these results are fragmentary, several points emerge from this work:

The proliferation of the skin epidermis is increased 24 h after plucking as demonstrated by the high labelling index.

At 24 h, the plucked skin has lost the inhibitory effect on epidermal DNA synthesis which is present in unplucked skin. After 4 days when the LI of the plucked skin is back to normal, the inhibitory effect of the skin has been regained. We intend to study epidermal hyperplasias which are not linked with plucking and human cutaneous proliferative diseases, such as psoriasis.

The experiments using tumour tissue fragments show two main points:

The epitheliomas of the C_3H mouse, which we studied, contain an inhibiting substance capable of affecting normal skin. Our results confirm the observations made, using different techniques, in other malignant epithelial tumours of the skin: rabbit VX2 carcinoma (Bullough & Laurence, 1968); Hewitt keratinizing epithelioma of the mouse (Bullough & Deol, 1971); and squamous-cell carcinoma of the hamster (Elgjo & Hennings, 1971) in which 'chalone-like substances' have been described.

Skin tumours of non epithelial nature also inhibit epidermal proliferation, the percentage of inhibition being similar to that found with normal skin and epitheliomas (Table 1). These results seem, at first sight, to be at variance with the known tissue specificity of the 'chalones' in normal skin and also in some ascitic tumours (Bichel, 1972) since the NCTC tumour 2472 (63% of inhibition) and the EMT6 tumour (53% of inhibition) are of mesenchymal nature. As for the mammary tumour we studied, developing at the expense of the stromal part of the gland and used after several transplantations in the mouse, it was histologically dedifferentiated without any recognizable epithelial elements. It therefore seems that some mesenchymal tumours contain a substance which inhibits epidermal proliferation. To explain this effect, one must take into consideration the fact that these tumours have the same histological appearance as the dermis and that the dermis, according to Elgjo & Edgehill's experiments (1973), contains a substance inhibiting the proliferation of epidermal cells. However, our results concern mainly experiments with tumours from skin and its components; experiments with tumours of other origin are envisaged since preliminary results obtained with a thymic lymphoma shows that its application on the ear wound did not inhibit epithelial proliferation (Table 1).

In conclusion, these studies demonstrate that plucked skin loses its inhibitory effect on cell proliferation when its labelling index is high and recovers the inhibitory effect when the labelling index returns to normal. Tumourous tissues of epithelial or mesenchymal origin inhibit DNA synthesis whereas preliminary data with a thymic lymphoma did not show this effect.

ACKNOWLEDGMENTS

We wish to thank Dr F.Loisillier from the "Laboratoire d'Anatomie Pathologique des Services Communs—Villejuif" for the histological work, Dr G.Barski, Dr I.Chouroulinkov and Mrs S. Rockwell who provided us with the tumours. This work was done with the help of INSERM.

REFERENCES

Bichel, P. (1972) Specific growth regulation in three ascitic tumours. *European Journal of Cancer*, **8**, 167.

Bullough, W.S. (1962) The control of mitotic activity in adult mammalian tissues. *Biological Reviews of the Cambridge Philosophical Society*, **37**, 307.

Bullough, W.S. (1973) The chalones: a review. *National Cancer Institute Monographs*, **38**, 5.

Bullough, W.S. & Deol, J.U.R. (1971) Chalone-induced mitotic inhibition in the Hewitt keratinizing epidermal carcinoma of the mouse. *European Journal of Cancer*, **7**, 425.

Bullough, W.S. & Laurence, E.B. (1960) The control of epidermal mitotic activity in the mouse. *Proceedings of the Royal Society, Series B*, **151**, 517.

Bullough, W.S. & Laurence, E.B. (1964) Mitotic control by internal secretion: the role of the chalone-adrenalin complex. *Experimental Cell Research*, **33**, 176.

Bullough, W.S. & Laurence, E.B. (1968) Control of mitosis in rabbit VX2 epidermal tumours by means of the epidermal chalone. *European Journal of Cancer*, **4**, 587.

Elgjo, K. (1969) Epidermal cell proliferation during the first 24 h after injection of an aqueous skin extract (chalone). *Virchows Archiv Abteilung B. Zellpathologie*, **4**, 119.

Elgjo, K. & Edgehill, W. (1973) Epidermal growth inhibitors (chalones) in dermis and serum. *Virchows Archiv Abteilung B. Zellpathologie*, **13**, 14.

Elgjo, K. & Hennings, H. (1971) Epidermal chalone and cell proliferation in a transplantable squamous cell carcinoma in hamster. I. *In vivo* results. *Virchows Archiv Abteilung B. Zellpathologie*, **7**, 1.

Elgjo, K., Laerum, O.D. & Edgehill, W. (1971) Growth regulation of mouse epidermis. I. G2 inhibitor present in the basal cell layer. *Virchows Archiv Abteilung B. Zellpathologie*, **8**, 277.

Elgjo, K., Laerum, O.D. & Edgehill, W. (1973) Growth regulation of mouse epidermis. II. G1 inhibitor present in the differentiating cell layer. *Virchows Archiv Abteilung B. Zellpathologie*, **10**, 229.

Finegold, M.J. (1965) Control of cell multiplication in epidermis. *Proceedings of the Society for Experimental Biology and Medicine*, **119**, 96.

Furth, J., Siebold, H.R. & Rathbone, R.R. (1933) Experimental studies on lymphomatosis of mice. *American Journal of Cancer*, **19**, 52.

Guigon, M., Frindel, E., Hewitt, J. & Tubiana, M. (1973) Etudes des chalones dans l'épiderme de la souris. *Annales de dermatologie et de syphiligraphie*, **100**, 279.

Houck, J.C. & Hennings, H. (1973) Chalones. Specific endogenous mitotic inhibitors. *Febs Letters*, **32**, 1.

Iversen, O.H., Bhangoo, K.S. & Hansen, K. (1974) Control of epidermal cell renewal in the Bat Web. A study of the cell number, cell size and mitotic rate in the epidermis on both sides of the web after the removal of the epidermis on the ventral side only, with special emphasis on growth control theories. *Virchows Archiv Abteilung B. Zellpathologie*, **16**, 157.

Le François, D., Youn, J.K., Belehradek, J. Jr & Barski, G. (1971) Evolution of cell-mediated immunity in mice bearing tumours produced by a mammary carcinoma cell line. Influence of tumor growth, surgical removal and treatment with irradiated tumour cells. *Journal of the National Cancer Institute*, **46**, 981.

Marks, R. (1972) The role of chalones in epidermal homeostasis. *British Journal of Dermatology*, **86**, 543.

Rockwell, S.C., Kallman, R.F. & Fajardo, L.F. (1972) Properties of transplantable mouse mammary tumour and its tissue-culture adapted derivative. *Journal of the National Cancer Institute*, **49**, 735.

Sanford, K.K., Likely, G.D. & Earle, W.R. (1954) The development of variations in transplantability and morphology within a clone of mouse fibroblasts transformed to sarcoma-producing cell *in vitro*. *Journal of the National Cancer Institute*, **15**, 215.

Yamaguchi, T. Hirobe, T., Kalinjo, Y. & Manaka, K. (1974) The effect of chalone on the cell cycle in the epidermis during wound healing. *Experimental Cell Research*, **89**, 247.

British Journal of Dermatology (1976) **95**, 137.

Sociological implications of an epidemiological study of eczema in the City of Birmingham

N.C.BOWKER,* K.W.CROSS,† E.A.FAIRBURN‡ AND M.WALL§

* Sociology Group, University of Aston, † Department of Hospital Statistics, Queen Elizabeth Medical Centre, ‡ Department of Diseases of the Skin, General Hospital, and §Statistics Department, West Midlands Regional Health Authority, Birmingham

Accepted for publication 23 November 1975

SUMMARY

An epidemiological study has been made of 4696 cases of eczematous dermatitis referred to hospital outpatient clinics in the City of Birmingham over a 3-year period. Some sociological implications of the investigation are discussed. The need for further sociological accounts of skin disease is stressed.

Investigation of the epidemiology and sociology of skin disease has been largely neglected. Although occasional insights emerge from the literature, they are usually very incomplete. However, several studies have shown that skin diseases make sizeable contributions to the total morbidity presenting at different levels of medical care.

Fry (1966) calculated that skin disorders comprised approximately 10% of the illnesses presenting in a London suburban general practice. This order of magnitude has recently been confirmed by a survey of over 100 general practices (O.P.C.S. 1974). Hall & Burrows (1968) reviewed the statistics of 157,381 new cases of skin disease seen in Northern Ireland hospitals from 1954 to 1966. They quoted the report of the medical officer in charge of the Queen's University Student Health Centre for the year 1965–66 in which it is shown that of 4500 students in the 18–24 age group, 1375 required medical attention during the academic year and of these, about one-third (445) sought advice because of skin disorders. Ratzen (1969) surveyed the dermatological outpatients of two large Glasgow hospitals from 1955 to 1964 and it was found that the 68,400 patients attending the skin departments comprised 8·3% of the total outpatient attendance of 816,700 patients.

These data suggest that there is a considerable National Health Service expenditure on dermatology and, indeed, the Office of Health Economics (1973) has estimated that in 1970 the direct quantifiable costs amounted to over £50 million.

Although conventional indices of public health, such as morbidity rates, define patterns of ill-health in populations, little is known concerning the factors leading individual patients to seek medical advice. Wadsworth, Butterfield & Blaney (1971) and Dunnell & Cartwright (1972) demonstrated the frequency of minor skin disease, much of which is not presented for clinical inspection. Their studies reveal, on the basis of the community's reporting of its own symptoms, that approximately 20% of people experience one or more symptoms in a 2-week reporting period.

Sociology and social psychology of medicine are largely silent regarding behavioural characteristics of sufferers from skin disease, apart from presenting usually inconclusive findings. There have been studies using the Rorschach test in the investigation of the body image of patients with atopic eczema and contact dermatitis (Eigenbrode & Shipman, 1960). These have proved indefinite and inconsistent.

The perception of various types of disability by the non-disabled has been studied and, in the context of American culture, skin disorders have appeared to evoke greater aversion than deafness, blindness or paralysis (Siller & Chipman, 1964). Such scattered findings illustrate the inadequacy of the existing basis for a systematic extra-clinical perspective of dermatology. However, the potentialities of a viewpoint which can furnish an adequate epidemiological and sociological account of derangements of the skin require to be recognized.

To attempt the compilation of a comprehensive dermatological history of a large population would be an over-ambitious and wasteful misuse of resources. However, an important component of the total reservoir of skin disorders which occur in a population over a given time is that which is referred to hospital for specialist attention. A preliminary study of the type of patient seen in dermatological outpatient clinics has previously been reported (Fairburn, Cross & Wall, 1970) and, in order to examine some of the above-mentioned issues, we have focused our attention upon those patients presenting with eczematous dermatitis, or 'eczema', which is a major segment of dermatological disease.

METHOD

Over a 3-year period we carried out a survey of the patients referred to dermatological hospital outpatient clinics in the City of Birmingham (population 1,064,000, in 1966). Although patients residing outside the city are seen at these clinics, such patients have been excluded from the study so that the findings may be related to the population of Birmingham. The methods of collection of data were given in the previous paper. Briefly, a record was completed for each patient attending for the first time the clinics of the six hospitals providing this service during the period 1965–68. The patient record, designed for computer analysis, included such details as: the consultant treating the patient, place of residence, age, sex, marital status, social class, ethnic group and occupation, in addition to the dermatological diagnoses.

The interpretation of the concept 'eczema' varies considerably amongst dermatologists. For the purposes of our study, gravitational eruptions and seborrhoeic dermatitis were excluded. Accordingly the data related to primary irritant dermatitis, allergic contact dermatitis, atopic dermatitis, nummular eczema, pompholyx and asteatotic eczema. The first two disorders were grouped under the title 'exogenous eczema', while the remainder were designated 'endogenous eczema'. This latter group comprises a heterogeneous collection of eczematous eruptions of unknown or partially known causation, but possessing in common the characteristic that they do not originate primarily as a cutaneous reaction to an external irritant or allergen. Finally, there remained those eczematous derangements of unspecified nature, which were designated 'not known'.

RESULTS

A total of 27,196 new patients attended during the period 1965–68, and of these 4696 (17%) presented with eczematous dermatitis. Table 1 shows the numbers of cases of eczema and the numbers of all dermatological cases treated by each of the eight consultant dermatologists providing outpatient services in the City of Birmingham. It will be noted that the proportion of eczema to all cases by consultant is fairly constant; there is, therefore, little divergence of opinion in the interpretation of what is and what is not an eczematous eruption. However, when the eczemas are divided into the three groups, considerable disparities appear. Proportions diagnosed as exogenous eczema ranged from 4 to 19%. Undoubtedly, this variation arises partly because eczemas are reaction patterns of skin and often implicate multiple aetiological factors, the determination of the relative importance of which is a matter of clinical practice rather than mathematical definition. Whether there are differences between individual consultants in referral patterns has not been pursued. Yet another possible factor relates to the very considerable range of waiting time for an appointment—from 1 to 4 months or thereabouts.

TABLE 1. Percentage distributions of all eczema patients by type of eczema (numbers of patients in parentheses) and proportions of eczema to all patients

Consultant	Exogenous eczema	Endogenous eczema	Eczema not known	Total eczema cases	Total skin cases	Eczemas as % of total
A	13	31	56	100 (1151)	6360	18
B	18	45	37	100 (709)	4182	17
C	13	79	8	100 (894)	5157	17
D	19	40	42	101 (632)	4221	15
E	17	40	42	99 (344)	1783	19
F	17	40	43	100 (183)	1003	18
G	12	53	36	101 (259)	1652	16
H	4	56	40	100 (458)	2478	19
Locum	17	45	38	100 (66)	360	18
Total	14 (661)	48 (2270)	38 (1765)	100 (4696)	27196	17

In such circumstances, it is to be anticipated that those eczematous disorders which ultimately may undergo spontaneous improvement would be more strongly represented in the clinics of a dermatologist with a short waiting list. The investigation of such possible differences illustrates the need to examine the overlap of epidemiological and sociological considerations.

Source of referral. The vast majority (92%) of all skin outpatients and of eczema cases were referred by general practitioners; 4 and 3% respectively were referred by consultants in other specialties; and only 0·6% of all skin and 0·3% of all eczema patients were referred by Works and School Medical Officers. To the extent that these figures reflect frequency of interaction between types of doctor and frequency of information exchange, the Works Medical Officers and School Medical Officers are largely isolated from dermatological outpatient practice, although there can be no doubt that a proportion of the patients initially attended the referring general practitioners on the recommendation of a physician at work or in the school.

Seasonal variation. The numbers of exogenous, endogenous and unknown eczemas by month of attendance at hospital were next obtained. The proportion which each of the three classes make to the total eczema cases each month, and the proportion of all eczemas to the total number of dermatological patients attending each month, are displayed diagrammatically in Fig. 1. It is evident that there is little monthly variation in the proportion of all eczemas to the total, although on the whole this is higher in the summer months, April to September. As regards exogenous eczemas, the percentage to all eczemas declines gradually from a peak value for February to a minimum in September, followed by an increase over the last 3 months of the year. The corresponding proportion of

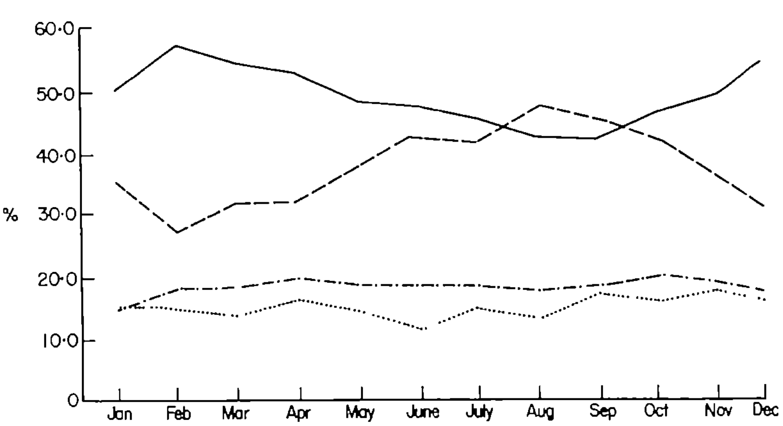


FIGURE 1. Proportion of all eczema patients to total dermatological patients, and proportions of the three classes of eczema patients to all eczema patients by month of attendance. —·— % total eczema to total skin cases; ——— % exogenous to total eczema; · · · · · · % endogenous to total eczema; – – – – % eczema not known to total eczema.

endogenous eczemas remains fairly constant over the 12 months. It is concluded that not only does the inter-consultant variation in waiting time render interpretation of these data of dubious value, but also a much greater knowledge of factors influencing hospital referral would be required before a meaningful attempt at explanation could be made.

Age and sex variations. The proportion of all eczemas to all dermatological patients was next examined by age group. The proportion was high (39%) for children under 5, and low (11%) for school children and young adults (15–24 years). After this age group the proportion gradually increased to 19% for the 55–64 years group, and then declined slightly to 17% for those patients 65 years and over. These results may well reflect the spontaneous lessening of atopic dermatitis which tends to occur after early childhood and is accompanied by a simultaneous increase in disorders such as warts and, rather later, acne vulgaris. Furthermore, variations in parental attitude require consideration: some mothers may reasonably believe that eczematous disorders are less critical to health among school children than in the very young.

When the proportions of exogenous eczemas to all eczema cases were considered by age group, it was found that the proportion was fairly high (16%) in the 'under 1-year-olds', doubtless due to the large number of napkin dermatitis cases encountered at this time of life. By contrast the proportion was very low (3%) among patients aged 1–14 years; and again this finding comes as no surprise to the clinician, since it is in childhood and adolescence that atopic dermatitis is especially common. Between 15 and 65 years, the proportion of exogenous eruptions gradually increased from 16 to 20%, and was followed by a fall to 13% in the elderly. It can be conjectured that this pattern reflects the interplay between occupational opportunities for exposure to external irritants and allergens and the gradual lowering of intrinsic resistance of the skin which occurs during the middle and later years of life.

In order to examine these aspects further, the numbers of cases by sex, age-group and type of eczema have been related to the population of Birmingham. Mean annual age- and sex-specific rates (per 100,000 population) are given in Table 2. For *all eczemas* the maximum rates for both males and females occurred in children under 5 years of age, the rates for school children being the lowest for males and second lowest for females; for males, rates gradually increase from that age group to a peak for the 55–64 years group, whereas for females high values were obtained for the age-groups 15–24 and 25–34 years and low values for the two oldest age groups.

TABLE 2. Mean annual age-sex specific rates per 100,000 population

Age group	Exogenous eczema		Endogenous eczema		Eczema not known		All eczemas	
	Males	Females	Males	Females	Males	Females	Males	Females
0–4	22	33	215	142	141	119	378	294
5–14	2	3	65	60	35	23	102	86
15–24	10	35	66	83	34	53	110	171
25–34	13	35	71	74	49	55	133	164
35–44	15	36	68	44	54	52	137	132
45–54	19	37	69	50	56	50	144	137
55–64	24	31	70	37	69	42	163	110
65+	15	13	58	27	76	43	149	83
All ages	14	27	81	61	59	52	154	140

As regards *exogenous* eczemas, the rate for males aged 5–14 years was very small and an increasing trend was evident from this age group up to 55–64 years; the female rate for the 5–14 years age group was also very small but for all other age groups, except the 65 years and over group, the rate was more or less the same.

Different patterns from these occurred for *endogenous* eczemas: for both males and females the pre-school children had the highest rates and the elderly the lowest; between 5 and 65 years the male rates remained more or less constant, whereas for females relatively high rates occurred in the 15–24 years and 25–34 years groups. On clinical grounds it would be expected to find high rates for endogenous eczemas in the elderly, but these did not occur, possibly because elderly patients often have difficulties in getting to hospital.

Finally, for eczemas of unknown aetiology the maximum rates for both males and females again occurred in the youngest age group; an increasing trend with age from the 15–24 years group was evident for males, whereas for females a low value occurred in the age group 5–14 years with values for the four age groups between 15 and 54 years all more or less the same as that for all ages combined.

TABLE 3. Percentage age distributions (all eczemas) for each ethnic group

Age group	European	West Indian	Indian	Pakistani	African	Other	All groups
<5	16·2	60·5	45·5	22·9	50·0	33·9	20·3
5–14	8·7	21·3	11·5	7·3	—	11·9	9·5
15–24	16·2	3·9	9·6	10·1	16·7	10·2	15·0
25–34	11·2	6·1	17·3	28·4	33·3	13·5	11·5
35–44	11·9	4·6	10·9	22·0	—	11·9	11·6
45–54	14·4	2·1	3·8	8·3	—	5·1	13·1
55–64	12·4	1·1	1·3	0·9	—	11·9	11·1
65+	9·0	0·4	—	—	—	1·7	7·9
Totals	100·0 (4071)	100·0 (281)	99·9 (156)	99·9 (109)	100·0 (6)	100·1 (59)	100·0* (4682)

* Excluding fourteen patients with unknown age or ethnic group.

Ethnic group variations. The influence of ethnic group was then considered and it was found that the proportion of all eczemas to the total skin cases varied from 11% for Africans (six cases only) to 29% for West Indians. The proportions for Indians and Pakistanis were 24 and 19% respectively as compared with 16% for Europeans. Probably these differences arise from the tendency for tropical races to develop xeroderma when resident in a cool temperate climate, since skin so affected is especially prone to eczematous derangements.

Table 3 shows that of the total West Indian eczema cases 60% were under the age of 5 years; and 45% of the Indian patients were in this age group. Half of all Pakistani eczema cases were in the age group 25–44 years, as compared with 23% of the European patients with eczema. These differences are partially due to differing age distributions of the immigrant groups, but they are sufficiently striking to suggest the operation of cultural in addition to biological differences between the age groups, possibly variations in treatment-seeking behaviour and in general practitioner–patient interactions. As regards the West Indian statistics, the social science literature calls attention to the highly maternal personalities of the women as a product of a socio-cultural history in which Caribbean men have suffered much unemployment or low status employment. This encourages dependent male behaviour and simultaneously markedly maternal female behaviour. Accordingly, it is interesting to conjecture whether these maternal women seek treatment for the eczematous problems of their preschool children more readily than do other mothers. Furthermore, are they speedier in seeking or prompting referral to an outpatient clinic?

Social class variation. Male eczema patients aged between 15 and 64 years in employment have been classified by social class, using the Registrar-General's classification of occupations. For each social class, the proportionate contribution of patients with each type of eczema to the total eczema patients is given in Table 4. No clear picture emerges from this analysis except that the proportion of 'not known' eczemas increased gradually from Class I to V; and this trend is offset by the tendency for the proportion of endogenous eczemas to decrease from Class I to V.

It seems probable that these are features of the communication and informational character of clinical interviews with Class V patients which present greater impediments to the formulation of a specific diagnosis than in the case of interviews with patients of higher social class. Moreover, it is possible that clinicians in certain situations make decisions guided by the patient rather than on the basis of medical need alone, and if this is indeed the case, it seems likely that the levels of minimum acceptability of care may vary according to the social class of the patient.

Referral rates by wards of Birmingham. In our previous communication, referral rates for patients with all skin disorders per 1000 population were calculated for the thirty-nine local government

TABLE 4. Percentage distributions of patients by type of eczema for each social class (numbers of cases in parentheses)

Social class	Exogenous eczema	Endogenous eczema	Eczema not known	Total eczema
I	14·6 (6)	56·1 (23)	29·3 (12)	100·0 (41)
II	18·4 (19)	49·5 (51)	32·1 (33)	100·0 (103)
III	10·0 (75)	53·3 (400)	36·7 (275)	100·0 (750)
IV	14·9 (43)	46·4 (134)	38·7 (112)	100·0 (289)
V	9·3 (12)	44·2 (57)	46·5 (60)	100·0 (129)
Total	11·8 (155)	50·7 (665)	37·5 (492)	100·0 (1312)

districts (wards) of Birmingham, and these were shown on a map of the city. This showed a clustering of relatively high rates in the central ward and in four adjacent wards to the west. Two of these had a mixed social class content, and in the remaining three, low standard housing was very common. Furthermore, it was noted that the outpatient clinics concerned in this survey were held in hospitals located in these wards. A significant correlation at the 5% level between ward referral rates and the percentage of persons at more than 1½ per room (in each ward) was reported although, of course, the location of the clinics may have accounted for this finding.

A similar analysis has been made of referral rates for eczema by ward of Birmingham: the ward rates for exogenous, endogenous, and all eczemas were related separately to each of ninety-four social indices available by ward and given in the Census Tables for 1966 (H.M.S.O.). For each type of eczema a series of correlation coefficients were computed which were tested for statistical significance. It should be mentioned that five coefficients would be found to be significant at the 5% level even if the null hypothesis of no association between any one of the 94 variables and the ward referral rates were true. Furthermore, several of these variables are themselves inter-related so that partial correlation coefficients were computed, taking in pairs those variables which were found to be significantly associated.

The following results of this correlation analysis were obtained. In the case of all eczemas it was found that there was a positive correlation with five social factors: (1) Percentage of total population born in Commonwealth and Colonies (excluding Australia, Canada and New Zealand). Our comments relating to the analysis by ethnic group are appropriate in this context. (2) Percentage of active and retired males who are in an unclassified social class. It is possible that a greater opportunity for attendance may be a factor in this group. (3) No children aged 0–4 years per 100 females aged 15–44 years. These women may have more time for seeking medical advice; or possibly there are other contributory factors such as subconscious seeking for more attention. (4) Percentage of households lacking a hot water tap. (5) Percentage of households lacking a fixed bath.

With regard to 4 and 5, from the medical standpoint it is hardly surprising that inadequate washing facilities and a consequent poor standard of skin hygiene is associated with an increased eczema referral rate. Moreover, it may be observed that very many forms of inequality can be correlated positively with low social class and these inequalities can be expected to impress themselves on behaviour, both physically and mentally in a variety of forms.

A negative correlation was demonstrated in the case of only one factor: women economically active. Thus it appears that the housewife is more likely to seek advice than her 'working' sister. This finding probably reflects either a heightened risk of exposure to potential cutaneous irritants, or a greater predisposition to anxiety and a consequent seeking of medical attention. In addition it may reflect a greater freedom for women to abandon employment than for men.

The remaining analyses were in large measure unrewarding. Thus, in the case of exogenous eczemas, no social factor correlated with referral rates; whilst as regards endogenous eczemas the only factor which correlated (positively) was 'percentage of active and retired males in an unclassified social class'; these men were retired or not employed, and in these circumstances had ample opportunity for introspection.

DISCUSSION

One object of epidemiology is 'to estimate from group experience what are the individual risks and chances, on average, of disease, accident and defect' (Morris, 1967). In these terms the epidemiological character of a hospital outpatient dermatological population is little illuminated by our eczema data. Nonetheless, the results of this necessary first stage of enquiry suggests the need to search for regularities in terms other than those which have often yielded relatively strong associations in the cases of various other diseases. A further aim of epidemiology is 'to complete the clinical picture of chronic disease and describe its natural history, by including in proportion all kinds of patients wherever they present, and by following the course of remission and relapse, adjustment and disability' (Morris, 1967). Here the implications of skin epidemiology are strongly sociological, since there is a dynamics of behaviour which underlies and influences such data as our Birmingham eczema statistics. We need to know more systematically about the remission and relapse of eczemas and other skin diseases, about how and to what extent these patterns reflect, for instance, failure to follow prescribed treatment, intra-familial interplays, the degree of tolerance of skin diseases of particular social groups as well as witting and unwitting environmental exposure. An element in the clinical picture which perhaps has particular relevance for skin cases is the pre-clinical and possibly simultaneous use of components of what Friedson (1961) calls the 'lay referral system'. Delay in presenting clinically, fallacies about skin conditions, and limitations on patient compliance might be traced to this source with consequences not only for case management and health education, but also for the planning of available medical services. What is needed is some systematic account of the sociology of skin diseases and of the ways in which specific sociological factors operate as components of the epidemiological and clinical pictures.

A strategic starting point could be an investigation aiming to describe and account for what people know, believe and do about skin diseases in a provincial city like Birmingham. For this purpose the population requires to be differentiated by appropriate social categories, with attention given to sources and reliability of knowledge of skin diseases and the variety of contexts, conditions and consequences of the learning process both before and after dermatological attention. Such study might be expected to yield information without which it remains difficult to make reliable inferences about the significance of rates of outpatient referrals, the efficacy and acceptability of treatment or the determination and definition of chronicity.

REFERENCES

DUNNELL, K. & CARTWRIGHT, A. (1972) *Medicine Takers, Prescribers and Hoarders*, p. 11. Routledge and Kegan Paul, London.

EIGENBRODE, C.R. & SHIPMAN, W.G. (1960) The body image barrier concept. *Journal of Abnormal and Social Psychology*, **60**, 450.

FAIRBURN, E.A., CROSS, K.W. & WALL, M. (1970) Some aspects of the dermatological outpatient population of Birmingham. *The Hospital*, **66**, 8.

FRIEDSON, E. (1961) *Patients' Views of Medical Practice: A Study of Subscribers to a Prepaid Medical Plan in the Bronx.* Russell Sage Foundation, New York.

FRY, J. (1966) *Profiles of Disease: A Study in the Natural History of Common Diseases*, p 9. E. and G. Livingstone, Edinburgh and London.

HALL, R. & BURROWS, D. (1968) Diagnostic review of hospital dermatology in Northern Ireland 1954–66. *British Journal of Dermatology*, **80**, 261.

MORRIS, J.N. (1967) *Uses of Epidemiology*. E. and G. Livingstone, Edinburgh and London.

OFFICE OF HEALTH ECONOMICS (1973) *Skin Disorders*. O.H.E., London.

O.P.C.S. (1974) *Morbidity Statistics from General Practice. Second National Study 1970–71*. Studies on Medicals and Population Studies No. 26, H.M.S.O.

RATZEN, M.A. (1969) The incidence of skin diseases in the west of Scotland. *British Journal of Dermatology*, **81**, 456.

SILLER, J. & CHIPMAN, A. (1964) Perceptions of physical disability by the non-disabled. Paper presented at *American Psychological Association meeting, Los Angeles, 1964*.

WADSWORTH, M.E.J., BUTTERFIELD, W.J.H. & BLANEY, R. (1971) *Health and Sickness: the Choice of Treatment*, p. 32. Tavistock Publications, London.

British Journal of Dermatology (1976) 95, 145.

Complement component profiles in urticaria, dermatitis herpetiformis, and alopecia areata

L.BERRENS, E.JANKOWSKI AND I.JANKOWSKI-BERNTSEN

Department of Dermatology, Division of Experimental Allergy, Academic Hospital,
Catharijnesingel 101-Utrecht, The Netherlands

Accepted for publication 2 December 1975

SUMMARY

Insignificant variation was noted in the mean levels of the complement components C1q, C4, C2, C3, C5 and C3PA between various groups of sera from patients with urticaria, angio-oedema, dermatitis herpetiformis, alopecia areata, or hay fever. No deficiencies were found. Elevated C9 levels in chronic urticaria and angio-oedema reflected its nature as an acute phase protein. The assessment of C components in single or sequential serum samples revealed only marginal variations. Similar results were recorded for plasminogen and the immunoglobulins G, A and M. Some evidence is provided for the primary involvement of the kallikrein-kinin system in urticaria.

A growing body of evidence suggests that the system of complement (C) factors occupies a key position in the mechanism of pathogenesis of many disease processes. A rationale for contemplating a possible role for C, even in those diseases which are not known to be associated with immune complexes, is provided by recent observations that a great many substances can engage the complement sequence in a non-immunological fashion. The determination of individual C components has provided some evidence for the direct or indirect involvement of the C system in various forms of urticaria. Heitman & Daum (1973) reported slightly elevated mean levels of the C components C3 and C4 in the sera of patients with chronic urticaria. Ballow *et al.* (1975a) recently described four patients with idiopathic urticaria or non-hereditary angio-oedema with low serum levels of C3; two of these patients had low serum values for haemolytic C1, C1q, C1s and properdin factor B (C3 Proactivator, C3PA), but the concentrations of C4 and C2 were normal. A group of 148 subjects with similar clinical disorders did not show these abnormalities. In contrast to cases of the hereditary form of angio-oedema, C1Inactivator levels (C1INA) were normal. In two female patients with chronic urticaria, Ballow and colleagues (1975b) detected significantly depressed levels of the C components C1q, C1s, $C\bar{1}$, C3, C8 and C9, whereas again C2 and C4 were normal.

Laurell, Mårtensson & Sjöholm (1975) recorded low C1q/C1s ratios in some cases of chronic urticaria; in six of sixty patients, C4 levels were reduced. In familial cold urticaria, Doeglas & Bleumink (1974) established normal serum levels of C3 and C4, which remained unaltered during cold exposure. Slight reduction of these C components was observed by Martens & Berrens (1975) during provocation in a case of essential acquired cold urticaria.

Although these results provide some indication of either primary or secondary involvement of the C system in urticaria, the phenomenon so far becomes apparent in only a limited number of patients. The question must be raised whether the determination of C factors in a single serum sample can be of assistance in the clinical and differential diagnosis other than in cases of clearcut deficiencies. The C system represents a vital cybernetic mechanism of specific and nonspecific defence, which is in a state of dynamic equilibrium; the assessment of C factors in an isolated sample therefore mirrors the balance between biosynthesis and consumption at the time the serum was taken.

We have examined the profiles of immunoglobulins and of individual C components in groups of patients with various forms of urticaria. In view of the suspected involvement of C in the atopy syndrome (Berrens, 1974), the sera of patients with seasonal hay fever were investigated for comparison. Patients with alopecia areata were included in the investigations, because the condition has been considered to feature a high incidence of atopy (Ikeda, 1965). Finally, the sera of patients with dermatitis herpetiformis were studied, as representative of a disease process for which there exists at least histological evidence for immune complex mediated C-engagement (Seah *et al.*, 1973).

MATERIALS AND METHODS

Plasmas and sera. Blood was obtained by venepuncture and either collected in citrate to provide platelet-poor plasma, or allowed to clot in glass to produce the serum. The samples were kept in small aliquots at −90°C until use. Patients providing the blood samples for investigation were either hospitalized subjects or individuals consulting the out-patient departments of dermatology and allergology. At the time of blood sampling, most patients had not received medication. None of the urticaria patients had a serum sickness type of illness. Immunoglobulins IgG, IgA, and IgM, plasminogen (Pg), α1-antitrypsin (α1-AT) and α2-macroglobulin (α2M) were determined by single radial immunodiffusion, using commercially available antiserum-agarose plates and standard protein solutions supplied by the manufacturer (Partigen, Behringwerke, Germany).

The *complement components* C1q, C4, C3, C3PA and C9 were similarly determined by single radial immunodiffusion, using either Partigen plates, if available, or gel plates specially prepared with antibody preparations obtained from Behringwerke. The factors C5 and C1INA were assayed by electroimmunodiffusion into agarose gel, containing dilute monovalent rabbit antiserum (Laurell, 1966). For C1q, C5 and C9 no standard proteins were available; in these cases, a reference serum was run as internal standard, composed of the pooled sera of four healthy individuals. In some cases, C1INA was also determined functionally by pH-stat assay, using partially purified C1$\bar{s}$ and N-acetyl-L-tyrosine-ethylester (ATEe) as substrate as described previously (Berrens & Guikers, 1972).

The *C2-component* of complement was determined by molecular titration of effective molecules as described by Borsos, Rapp & Mayer (1961), using sheep erythrocytes E, rabbit amboceptor A and human euglobulin precipitate to produce the required EAC$\overline{1,4}$ cellular intermediates ($7{\cdot}5 \times 10^7$ cells per test). Total haemolytic complement was assayed with $2{\cdot}5 \times 10^8$ cells according to Kabat & Mayer (1964) and was expressed in CH50 units/ml serum.

RESULTS

The serum profiles of immunoglobulins and C components in none of our cases of urticaria revealed complete deficiency of any of the investigated factors. The levels of immunoglobulins G, M and A were quite normal, although in some cases of the physical urticarias low IgA values were recorded. In a few cases of acute urticaria, and particularly in acute angio-oedema, C9 levels were considerably elevated, but this was also found in non-urticarial diseases in the acute stage (e.g. Case 14, Table 1, pyoderma gangrenosum). Similarly, C5 levels were often elevated in active disease processes, but this phenomenon was not associated with a particular diagnosis. As shown by a few selected examples in Table 1, the C component and immunoglobulin profile of individual sera gave little indication of abnormalities or ostentatious deficiencies.

As regards plasminogen, it is of some interest that this precursor enzyme was decreased in the case of exercise-induced urticaria (Case 7, Table 1). The low value is indicative of depletion by active fibrinolysis, a process known to accompany exercise in general (McAlpine, Milojevic & Monkhouse, 1971).

As expected from the results with individual sera, the mean value for the immunoglobulins G, M and A, and for the various C components did not differ among the different forms of urticaria. Nor did the mean values of these serum proteins differ significantly from the average levels established in dermatitis herpetiformis, alopecia areata, or hay fever (Tables 2 and 3). As shown in Tables 2 and 3, the mean values were within the normal range established with the sera of healthy blood donors.

In contrast to reported cases of hereditary angio-oedema, none of the sera of our group of patients with acquired angio-oedema had a pronounced deficiency or a depressed level of C_1 esterase inhibitor (C1INA) (Table 1). This was true for the structural values established by electro-immunodiffusion (Laurell, 1966), but it was also found for inhibitor levels determined by functional assay of $C\bar{1}$ esterase inhibition by a pH-stat technique (Berrens & Guikers, 1972). Under standard conditions, the percent inhibition of a fixed amount of $C\overline{1s}$ on average was 50% (N = 58, s.d. = 10%), against 43% for a group of twenty-one patients with angio-oedema (s.d. = 13%).

Low C3 levels, as reported by Ballow *et al.* (1975a, b) and by Webb, Pearsall & Sumida (1975) in some cases of chronic urticaria or angio-oedema of unknown aetiology, were not detected in this study (Table 3). Normal mean values of C3 and C4 were found in chronic urticaria (Table 3), in contrast to the results reported by Heitman & Daum (1973).

Serum levels of C1q ranged about normal in groups of patients with chronic urticaria (mostly of unknown aetiology), acquired angio-oedema or cholinergic urticaria (Fig. 1). C5 levels in these groups of patients displayed a wider range; values above or below normal were apparently unrelated to the disease stage or to therapy. In both chronic urticaria and angio-oedema, C9 levels tended to be well above normal (Fig. 1). This may reflect the involvement of C9 as an acute phase protein rather than overproduction of the entire system of complement factors by positive feed-back biosynthesis, because elevated C9 levels were not correlated with levels of other C factors.

According to Kawachi-Takahashi *et al.* (1975), C9 is often elevated in the serum of patients with infectious or allergic skin disease and as such demonstrates a positive correlation with another acute phase α-globulin, α1-antitrypsin. Our results confirm that an elevated C9 level may reflect an uncontrolled active disease process; in one case of angio-oedema, the C9 value dropped from 204% of normal in the acute phase to 143% in the quiet phase. In two cases of chronic urticaria of unknown aetiology, C9 levels remained at 230% of normal in follow-up sera, indicating that the triggering cause of the disease had not been found.

The C2 component of complement occurs at very low concentration in the serum and normally is the rate-limiting factor in the sequence of complement reactions activated through the 'classical' pathway, i.e. by C1-activation. Functional assays of C2 in some cases of non-hereditary angio-oedema and in dermatitis herpetiformis showed normal mean C2 values (Table 4). Taken together with the normal mean values for C1q (Fig. 1) and of C4 and C3 (Table 3), this could be interpreted to mean unimpaired functioning of the classical pathway of complement activation in these two diseases.

Dermatitis herpetiformis has been considered a condition caused by activation of the 'alternate' pathway of C-consumption, bypassing the early C components C1, C4 and C2. Activation of C3 in this case is thought to occur by immune complexes of IgA antibody and unknown antigen and to involve the participation of factors of the properdin system, where C3PA (Factor B) occupies a key position. The hypothesis is largely based on the detection of IgA and alternate C factor deposits in skin sections by immunofluorescence techniques (Seah *et al.*, 1973). Our results on IgA, C3 and C3PA listed in Tables 2 and 3 show that such a possible mechanism in any event is not reflected in altered serum levels of the key components.

Average values for the investigated immunological parameters in alopecia areata and hay fever did not deviate significantly from those obtained in healthy controls (Tables 2 and 3).

On the whole, the single determination of complement and immunoglobulin components in the serum of patients with various forms of urticaria proved very unrewarding. It was therefore considered that the longitudinal assessment of these complex systems during provocation might provide better

TABLE 1. Immunological parameters in a few selected cases of urticaria and other dermatological disorders

No.	Diagnosis	IgG	IgA	IgM	Plasminogen	C1q	C4	C3	C3PA	C5	C9	C1INA
1	Cholinergic urticaria	820	38	196	21·0	118	57·0	104	46·0	150	264	46·8
2	Aquagenic urticaria	1040	213	115	12·0	104	27·0	72	16·8	70	90	33·2
3	Pressure urticaria	820	83	120	19·8	74	33·2	68	15·8	50	114	22·6
4	Delayed pressure urt.	1630	310	216			35·2	95	25·2			25·6
5	Chronic urticaria	1080	184	150	10·6	114	40·8	104	33·6	132	228	37·6
6	Acute urticaria	1070	162	179	23·6	114	39·6	92	36·0	134	162	46·0
7	Exercise-induced urt.	1040	58	88	8·6	86	26·0	64	13·8	32	58	22·2
8	Acquired cold urticaria	1680	224				31·6	76	19·2			28·8
9	Acute angio-oedema	1070	162	179	23·6	114	39·6	92	36·0	134	162	46·0
10	Dermatitis herpetiformis	1500	342	112			45·6		22·0			
11	Alopecia areata	1680	272	88			75·2	84	22·4			29·5
12	Erythropoietic protoporphyria	1400	276	92			30·8		19·2			35·1
13	Lyell's disease	1675	328				26·0	98	24·0			41·6
14	Pyoderma gangrenosum	535	86	206	14·6	100	60·0	114	42·8	200	400	72·0

Values in mg/100 ml; C1q, C5 and C9 in percent of normal.

TABLE 2. Immunoglobulins and plasminogen in the sera of patients with various forms of urticaria, as compared to dermatitis herpetiformis, alopecia areata, and hay fever

	IgG			IgA			IgM						Plasminogen		
Disease group	N	Mean ± s.d.	Range	N	Mean ± s.d.	Range	N (♂)	Mean ± s.d.	Range	N(♀)	Mean ± s.d.	Range	N	Mean ± s.d.	Range
Urticaria, whole group	48	1178 ± 271	820–1750	48	202 ± 99	38–460	18	143 ± 69	68–364	23	153 ± 38	68–233	38	13·1 ± 5·3	8·0–35·6
Chronic urticaria	13	1179 ± 224	820–1750	13	205 ± 105	77–460	13 (♂)	166 ± 71	77–364				12	12·1 ± 2·6	9·2–16·6
Cholinergic urticaria	8	1123 ± 248	820–1480	8	199 ± 71	83–282	7 (♂)	151 ± 27	115–167				7	14·0 ± 9·6	8·8–35·6
Angio-oedema/ urticaria	16	1082 ± 247	690–1455	16	176 ± 86	38–312	8	128 ± 45	68–202	5	173 ± 46	68–233	14	13·3 ± 4·8	8·6–23·6
Dermatitis herpetiformis	18	1689 ± 352	1300–2420	18	306 ± 81	174–750	18 (♂)	125 ± 40	70–206				N.D.	N.D.	N.D.
Alopecia areata	19	1467 ± 214	1020–1820	19	205 ± 79	100–416	5	159 ± 43	106–204	11	146 ± 56	88–258	N.D.	N.D.	N.D.
Normal values		1200	800–1800		210	90–450		90	60–250		110	70–280		20	10–30
Coefficient of variation		5·4%			2·7%			3·0%			3·0%			1·5%	

Values in mg/100 ml. N indicates the number of samples.

TABLE 3. Complement component profiles in the sera of patients with various forms of urticaria, as compared to dermatitis herpetiformis, alopecia areata, and hay fever

	C4			C3 (β1c)			C3PA			C1INH		
Disease group	N	Mean ± s.d.	Range	N	Mean ± s.d.	Range	N	Mean ± s.d.	Range	N	Mean ± s.d.	Range
Urticaria, whole group	48	35·2±11·0	15·6–57·0	48	85±15	60–126	48	22·5±6·2	13·8–36·0	65	31·4±7·3	17·2–46·8
Chronic urticaria	13	34·0±11·0	19·6–52·4	13	83±16	60–108	13	22·2±6·0	13·8–36·0	12	33·3±8·2	24·0–49·2
Cholinergic urticaria	8	33·8± 9·5	19·6–52·8	8	76±10	68– 92	8	20·4±4·5	16·8–26·0	8	26·1±4·2	22·0–37·8
Angio-oedema/ urticaria	16	37·4±11·7	15·6–57·0	18	85±15	66–120	16	24·3±8·0	13·8–46·2	35	33·0±7·5	17·2–47·2
Dermatitis herpetiformis	18	32·9± 7·9	22·4–47·4	N.D.	N.D.	N.D.	18	20·8±4·6	12·4–26·0	N.D.	N.D.	N.D.
Alopecia areata	18	33·6±14·2	18·0–75·2	17	79±20	48–116	16	21·9±3·0	18·8–28·0	17	30·1±3·3	23·2–33·3
Hay fever	33	32·6±10·0	14·0–57·2	N.D.	N.D.	N.D.	33	19·5±3·1	13·6–24·8	32	30·5±3·1	24·4–35·9
Normal values		30	20–50		82	55–120		22·0	20–25		25	15–35
Coefficient of variation		2·4%			5·7%			4·0%			3·7%	

Values in mg/100 ml. N indicates the number of samples.

TABLE 4. Values for functional C2 component in dermatitis herpetiformis and in angio-oedema/urticaria determined by molecular titration of effective molecules (Borsos *et al.*, 1961)

Disease group	N	Mean ± s.d.	Range
Dermatitis herpetiformis	8	2180±379	1760–2920
Angio-oedema	16	1820±475	1160–2440
Normal values		2000	1500–2500

Values in C2H50/ml; N indicates number of samples.

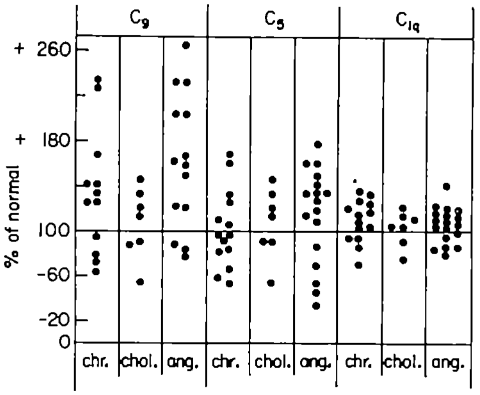


FIGURE 1. Levels of the C components C1q, C5 and C9 in the serum of patients with chronic (chr.), cholinergic (chol.) urticaria and in forms of urticaria with acquired angio-oedema (ang.). Values in percent of a normal serum pool. Coefficients of variation: 5·6% (C1q), 2·4% (C5), 4·3% (C9). Normal range of absolute values 10–25 mg/100 ml (C1q), 4–15 mg/100 ml (C5), 0·1–1·0 mg/100 ml (C9).

TABLE 5. Immunoglobulins, complement components and protease inhibitors in a case of factitious urticaria and a case of cold urticaria, established before and after provocation

Plasma component	Factitious urticaria		Cold urticaria	
	Before	After	Before	After
IgG	640	660	1320	1410
IgA	36	36	264	290
C4	24·8	24·0	26·0	30·4
C3	70	72	76	92
C3Proactivator	15·2	14·4	12·8	15·6
α1-Antitrypsin	130	160	40	268
α2-Macroglobulin	296	334	215	170
C1-esterase inhibitor	26·6	27·4	226·6	28·6
CH50	60	58	46	54

All values in mg/100 ml; total haemolytic complement in CH50/ml.

insight into the functioning of mediator systems in urticaria. Table 5 lists two examples. In one case of acquired cold urticaria, some complement factors and enzyme inhibitors were determined in the serum before immersion of the forearm in cold water (8°C), and at the height of the urticarial reaction 20 min later. All values were slightly, though not significantly increased, with the exception of α2-macroglobulin (Table 5). Insignificant variation was similarly noted in a case of factitious urticaria, provoked by deliberate rubbing of the back (Table 5). In the latter case, however, a dramatic change occurred in the blood clotting system. Before provocation, the recalcification time of the platelet-poor plasma in siliconized tubes was 12 min 10 s; 20 min after provocation, the clotting time was reduced to 4 min 17 s. This indicates involvement of Hageman factor-prekallikrein-plasminogen systems rather than the complement system. Activation of plasminogen by provocation in factitious urticaria was confirmed by observing fibrinolysis: the euglobulin clot lysis time fell from 190 min before challenge to 120 min after challenge.

DISCUSSION

The assessment of complement factors in the present studies has demonstrated that single determinations of C components in the sera of urticaria patients is of no value in diagnosis or differential diagnosis. Although in individual cases elevated or depressed levels of C factors or immunoglobulins were found, no relationship existed between quantitative variation and the nature or severity of the urticarial condition. The discovery of low levels of individual components in some isolated cases, as described for C1q (Laurell *et al.*, 1975) and for C3 and C4 (Ballow *et al.*, 1975a, b; Heitman & Daum, 1973; Webb *et al.*, 1975) must therefore be accidental rather than pathognomonic of particular urticarial reactions. The only C component often found elevated was the C9 factor, especially in chronic urticaria and in acquired angio-oedema. As an acute phase protein, C9 proved a valuable indicator of the acuteness of the patient's condition and as a reflection in general of ongoing inflammatory processes. We gained the general impression, already expressed by Schutte *et al.* (1974, 1975), that the C system as a whole, with the exception of the properdin factor, responds as an acute phase reactant during inflammatory disease. Consequently, the assessment of the C system or its multiple individual components in urticaria may have some value only in longitudinal series, e.g. as a function of provocation or therapy. The examples shown in this study, of factitious and cold urticaria, however, are discouraging because of only marginal variation in the circulating level of some key complement factors.

The response of the C system in urticaria in general appears to be of a secondary nature. The primary sensitiveness in some forms of urticaria seems to be located in the kallikrein-kinin system and its intricate relation to the early stages of the intrinsic clotting system. Dramatic changes in clotting time were observed after provocation in a case of factitious urticaria included in this study (Table 5). Prekallikrein or Factor XII hyperactivation by kaolin *in vitro* has recently been demonstrated in a case of acquired essential cold urticaria (Martens & Berrens, 1975). Our unpublished evidence so far indicates that this may be a general rule in acquired cold urticaria. The *in vivo* involvement of the kallikrein, rather than the complement, system in urticaria is sustained by the reported clinical results with therapeutic kallikrein inhibitors (Berova, Petkov & Adreev, 1974; Juhlin & Michaëlsson, 1969).

REFERENCES

Ballow, M., Ward, G.M., Gershwin, M.E. & Day, N.K. (1975b) C1-bypass complement-activation pathway in patients with chronic urticaria and angio-oedema. *Lancet*, **ii**, 248.

Ballow, M., Ward, G.M., Wilbur, R. & Day, N.K. (1975a) Abnormal serum complement profile in patients with idiopathic urticaria. *Journal of Allergy and Clinical Immunology*, **55**, 105.

Berova, N., Petkov, I. & Adreev, V.C. (1974) Treatment of chronic urticaria with a proteinase (kallikrein) inhibitor. *British Journal of Dermatology*, **90**, 431.

Berrens, L. (1974) Inhalant allergens in human atopic disease: their chemistry and modes of action. *Annals of the New York Academy of Sciences*, **221**, 183.

Berrens, L. & Guikers, C.L.H. (1972) An immunochemical study of pigeon-breeder's disease. III. The interaction of pigeon intestinal enzyme with human serum and a study of serum inhibitors. *International Archives of Allergy*, **43**, 337.

Borsos, T., Rapp, H.J. & Mayer, M.M. (1961) Studies on the second component of complement. I. The reaction between EAC′1,4 and C′2: evidence on the single site mechanism of immune hemolysis and determination of C′2 on a molecular basis. *Journal of Immunology*, **187**, 310.

Doeglas, H.M.G. & Bleumink, E. (1974) Familial cold urticaria. *Archives of Dermatology*, **110**, 382.

Heitman, H.J. & Daum, H. (1973) Quantitative Bestimmung von β1A- und β1E-Globulinen in Blutserum bei der Urticaria chronica. *Hautarzt*, **24**, 118.

Ikeda, T. (1965) A new classification of alopecia areata. *Dermatologica*, **131**, 121.

Juhlin, L. & Michaëlsson, G. (1969) Use of a kallikrein inhibitor in the treatment of urticaria and hereditary angioneurotic edema. *Acta dermato-venereologica*, **49**, 37.

Kabat, E.A. & Mayer, M.M. (1964) *Experimental Immunochemistry*, 2nd edn. Charles C.Thomas, Springfield.

Kawachi-Takahashi, S., Tanaka, K., Takahashi, M., Kawashima, T. & Shimada, K. (1975) Determination of serum C9 level by immunodiffusion. Elevation in patients with infections or allergic skin disease. *International Archives of Allergy*, **48**, 161.

Laurell, A.-B., Mårtensson, U. & Sjöholm, A.G. (1975) Complement components in hereditary angioedema and chronic urticaria. *International Archives of Allergy*, **49**, 86.

Laurell, C.-B. (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analytical Biochemistry*, **15**, 45.

Martens, B.P.M. & Berrens, L. (1975) Enzyme activation and inhibition induced by cold provocation in a patient with cold urticaria. *Acta dermato-venereologica*, **55**, 121.

McAlpine, V., Milojevic, S. & Monkhouse, F.C. (1971) Changes in the electrophoretic patterns of euglobulin fractions following activation of the fibrinolytic system by exercise. *Canadian Journal of Physiology and Pharmacology*, **49**, 672.

Schutte, M., Dicamelli, R., Murphy, P., Sadove, M. & Gewurz, H. (1974) C3Proactivator (C3PA) as an acute phase reactant. *Clinical and Experimental Immunology*, **18**, 251.

Schutte, M., Dicamelli. R., Murphy, P., Sadove, M. & Gewurz, H. (1975) Effects of anesthesia, surgery and inflammation upon host defence mechanisms. I. Effects upon the complement system. *International Archives of Allergy*, **48**, 706.

Seah, P.P., Fry, L., Mazaheri, M.R., Mowbray, J.F., Hoffbrand, A.V. & Holborow, E.J. (1973) Alternate pathway complement fixation by IgA in the skin in dermatitis herpetiformis. *Lancet*, **ii**, 175.

Webb, D.R., Pearsall, H.R. & Sumida, S.E. (1975) Depression of third component of complement (C3) in chronic angioedema. *Journal of Allergy and Clinical Immunology*, **55**, 106.

British Journal of Dermatology (1976) **95**, 153.

Pilo-sebaceous duct physiology

III. OBSERVATIONS ON THE NUMBER AND SIZE OF PILO-SEBACEOUS DUCTS IN ACNE VULGARIS

W. J. CUNLIFFE, W. D. H. PERERA, P. THACKRAY, M. WILLIAMS,
R. A. FORSTER AND S. M. WILLIAMS

Departments of Dermatology, The General Infirmary, Leeds, and
Leeds (St James's) University Hospital

Accepted for publication 14 November 1975

SUMMARY

We have used surface microscopy to measure the pilo-sebaceous duct orifices at different sites in 150 subjects. Our data have shown that there are more pilo-sebaceous units on the face compared to the back and that these exits are smaller on the face. Despite the small exit the sebum excretion rate per gland on the forehead was not significantly different from that on the back. Thus the number of pilo-sebaceous duct units and the pilo-sebaceous canal exit size are possible factors in the localization of acne.

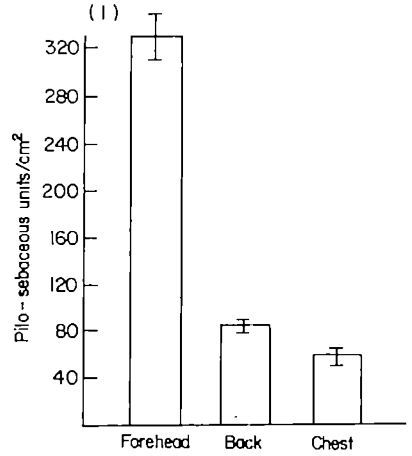
The principal factors in the pathogenesis of acne are an enhanced sebum excretion rate (Cunliffe & Shuster, 1969a), variations in the biochemical composition of sebum (Powell & Beveridge, 1970: Cotterill *et al.*, 1972; Peter & Eichenscher, 1973) caused to some extent by bacterial enzymes (Marples, Downing & Kligman, 1971), and sebum outflow resistance (Cunliffe & Shuster, 1969a). Acne is most evident on the face and there are several reasons why this could be so. First, there could be an enhanced response of the sebaceous gland to androgens, probably involving an increased rate of conversion of testosterone to its more active metabolite (Sansone & Reisner, 1971). Secondly, there may be an increased sebum outflow resistance on the face compared to the other sites. Plewig (1974) has demonstrated an increased epithelial turnover in the pilo-sebaceous canal on the face compared to the back. Kligman (1974) has shown that the keratin in the facial pilo-sebaceous canals is softer and more pliable than the rigid keratin found in the canals on the back. Furthermore, acne is uncommon in the open-mouthed follicles of the nose, and large inflammatory spots are more frequently seen arising from closed comedones; the inference being that the acne may be less evident in areas with larger pores. We therefore thought it worthwhile looking at the size of the pilo-sebaceous ducts in an attempt to explain the localization of acne.

MATERIALS AND METHODS

We have investigated a total of 150 subjects with acne. One hundred and thirty-nine had acne of a similar degree on the face and back; eleven had no acne on the back. The subjects investigated represented a fairly homogeneous population and were selected for the various tests simply by their attendance at the acne clinic. No patient was on therapy at the time or for 4 weeks before the study. Measurements of the pilo-sebaceous duct orifice were made at three sites: the mid-point of the forehead; the chest 2.5 cm below the sternal angle; and the upper back. A template was used to ensure that the same area of the back was read in each individual; two medial and lateral areas were chosen. The method of measuring, which has been described elsewhere (Cunliffe, Forster & Williams, 1974), involves staining the skin with Oil Red O and viewing with a Leitz MZ microscope (×50 magnification). A graticule incorporated into the eyepiece allows the diameter of the pilo-sebaceous duct exit to be measured. We also measured the sebum excretion rate from the forehead by the method of Strauss & Pochi (1961) modified by Cunliffe & Shuster (1969b). Despite certain minor technical problems we were able to collect sebum from the back, particularly if we chose an area slightly medial to the scapula, the paper being held in position by a blue lined bandage fixed with nylon mesh over the anterior chest.

RESULTS

During the study we obtained data on the number of pilo-sebaceous ducts at all sites. We found (Fig. 1) significantly more ducts on the forehead compared to the back and in turn there were many more ducts on the back compared to the chest. The pilo-sebaceous exits on the forehead were significantly smaller than on the back and those on the back were smaller than those on the chest (Fig. 2). We also compared the number and size of the pilo-sebaceous duct exits on the medial and lateral parts of the back. There were significantly more ducts on the upper back laterally and these ducts were significantly smaller than on the medial part of the back (Table 1). Fig. 3 shows the sebum excretion rate per duct per min. Although there was some difference in four subjects, there was no overall significant difference between the sebum excretion rate per gland on the forehead and back.



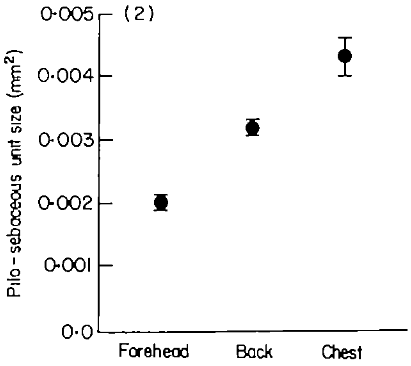


FIGURE 1. The number (mean ± s.e. mean) of pilo-sebaceous units on the forehead, upper back and anterior chest. 120 subjects were investigated. Back, N = 47, $P < 0{\cdot}01$; Chest, $P < 0{\cdot}01$.

FIGURE 2. The pilo-sebaceous exit size (mean ± s.e. mean) on the forehead, upper back and anterior chest. Forehead, $P < 0{\cdot}001$; Back, N = 47, $P < 0{\cdot}001$.

DISCUSSION

We have confined this study to the forehead, back and chest since these are the sites usually involved in acne vulgaris. Our data have shown that there are considerably fewer pilo-sebaceous units on the back than on the forehead and even fewer on the chest. It is clear from this result that the forehead is at greater risk than the back or chest as the number of ducts per unit area is higher. Our study did not include the scalp, as the scalp, probably because of the terminal hair follicles, is not involved in acne.

Our absolute counts are similar to those reported by Benfenati & Brillianti (1939) and Powell & Beveridge (1970) who investigated the number of ducts on the face by an indirect method. These

TABLE 1. The number and size of the pilo-sebaceous ducts on the lateral and medial parts of the back (mean ± s.e.mean)

	Medial	Lateral	No. of subjects	P
No./cm^2	72·0 ± 2·0	84·6 ± 3·6	35	< 0·01
Size (mm^2)	0·0030 ± 0·0002	0·0039 ± 0·0002	65	< 0·01

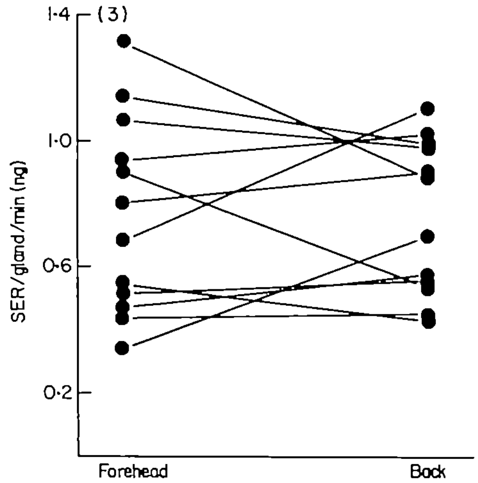


FIGURE 3. The sebum excretion rate per gland per min on the forehead and upper back. $P <$ 0·98; Forehead, $\bar{x}$ = 0·70; Back, $\bar{x}$ = 0·71.

authors used osmium tetroxide to visualize the number of fat droplets on collecting paper. It is perhaps surprising that both methods show the same result since the indirect method, at least in our hands, gives variable results. On the other hand, we are not entirely certain whether we are counting only the sebaceous follicles (the main type of follicle involved in acne, Kligman, 1974) or whether we are also counting the smaller vellus follicles. There is no doubt, however, that the number of ducts we count does not vary during the investigation; nor is there any variation in the area of the exit during the measurement. This direct method is reproducible and the Oil Red O probably stains the lipid bound keratin—enabling us to measure accurately the size of the pilo-sebaceous exit. We realize that some patients have acne predominantly localized to the back rather than to the face and we appreciate that there must be other factors localizing acne to different sites, such as variations in local androgen metabolism or in the cellular dynamics of the pilo-sebaceous keratin (Baillie, Thompson & Milne, 1966; Plewig, 1974).

Poiseuille's formula (Noakes, 1963), which relates the outflow resistance in a simple tube to flow properties, indicates that the resistance is inversely proportional to the fourth power of the radius and this is in contrast to other factors which vary only linearly. Thus it is reasonable to assume that in the pilo-sebaceous canal, although it is not a simple tube, the resistance to the outflow of sebum will vary with the radius of the duct orifice. Therefore, the smaller the pilo-sebaceous duct orifice the higher the resistance.

It would therefore appear that the localization of acne may also be explained by the size of the pilo-sebaceous duct exit; the smaller duct exits, such as are seen on the forehead, being related to a higher prevalence of acne at that site.

This hypothesis may not necessarily be valid for it could be argued that through the smaller ducts on the forehead only a small amount of sebum is excreted, whereas the larger ducts on the back may convey proportionally larger amounts of sebum. However, our sebum excretion rate studies show that the same volume of sebum flows through the much larger ducts on the back. Assuming that there is no difference in the viscosity of sebum or in the length of the pilo-sebaceous canals, calculation of the outflow resistance from Poiseuille's formula shows that it is five times greater on the forehead than it is on the back. Where the outflow resistance is greater such as on the forehead the pilo-sebaceous units are therefore more likely to become obstructed especially under such conditions as keratin hydration, which is known to reduce significantly the pilo-sebaceous exit size (Williams, Cunliffe & Gould, 1974).

We have therefore demonstrated that the localization of acne can to some extent be explained by both the number of the pilo-sebaceous units and the size of the pilo-sebaceous exit. This fits well with the clinical observation that acne is uncommon in areas where the pores clinically appear larger, such as on the medial part of the back, the medial part of the cheek and the nose.

ACKNOWLEDGMENTS

We wish to thank Vick International and the Leeds Area Health Authority for their financial support. We also wish to thank Mrs L.Lane and Mrs J.Hodson for typing the manuscript.

REFERENCES

Benfenati, A. & Brilliant, F. (1939) Sulla Distribuzione delle Ghiandole Sebacee nella cute del corpo humano. *Archivio italiano di dermatologia, sifilografia e venereologia*, **15**, 33, quoted by J.S.Strauss & P.E.Pochi (1968) in: *Jadassohn's Handbuch der Haut- und Geschlechtskrankheiten Erganzungwerk*, Vol. I, Pt. 1 (Ed. by O.Gans and G.K.Steiglcder), p. 184. Springer, Berlin.

Baillie, A.H., Thompson, J. & Milne, J.A. (1966) The distribution of hydroxysteroid dehydrogenase in human sebaceous glands. *British Journal of Dermatology*, **78**, 451.

Cotterill, J.A., Cunliffe, W.J., Williamson, B. & Bulusu, L. (1972) Further observations on the pathogenesis of acne. *British Medical Journal*, **iii**, 444.

Cunliffe, W.J. & Shuster, S. (1969a) Pathogenesis of acne. *Lancet*, **i**, 685.

Cunliffe, W.J. & Shuster, S. (1969b) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697.

Cunliffe, W.J., Forster, R.A. & Williams, M. (1974) A surface microscope for clinical and laboratory use. *British Journal of Dermatology*, **90**, 619.

Kligman, A.M. (1974) An overview of acne. *Journal of Investigative Dermatology*, **62**, 268.

Knutson, D.D. (1974) Ultra structural observations in acne vulgaris; the normal sebaceous follicle and acne lesions. *Journal of Investigative Dermatology*, **62**, 288.

Marples, R.R., Downing, D.T. & Kligman, A.M. (1971) Control of free fatty acids on human surface lipid by *Corynebacterium acnes*. *Journal of Investigative Dermatology*, **56**, 127.

Noakes, G.R. (1963) *New Intermediate Physics*, Macmillan, London.

Plewig, G. (1974) Follicular keratinisation. *Journal of Investigative Dermatology*, **62**, 308.

Perera, W.D.H., Cunliffe, W.J., Williams, S. & Edwards, J.C. (1976) Assessment of density of pilo-sebaceous duct on the forehead: a comparison of methods. *British Journal of Dermatology*, in press.

Peter, G. & Eichenscher, K. (1973) Relationen in der Lipidzusammensetzung von Comedonen, palmarer Hautoberflache and Talgdrusen. *Archiv für Dermatologische Forschung*, **247**, 329.

Powell, E. & Beveridge, G.W. (1970) Sebum excretion and sebum composition in adolescent men with and without acne vulgaris. *British Journal of Dermatology*, **82**, 243.

Sansone, G. & Reisner, R.M. (1971) Differential rate of conversion of testosterone to dihydrotestosterone in acne and normal human skin—a possible pathogenic factor in acne. *Journal of Investigative Dermatology*, **56**, 366.

Strauss, J.S. & Pochi, P.E. (1961) The quantitative gravimetric determination of sebum production. *Journal of Investigative Dermatology*, **36**, 243.

Williams, M., Cunliffe, W.J. & Gould, D. (1974) Pilo-sebaceous duct physiology. 1. Effect of hydration on pilo-sebaceous duct orifice. *British Journal of Dermatology*, **90**, 631.

British Journal of Dermatology (1976) **95**, 157.

Erythropoietic protoporphyria

HEPATIC IMPLICATIONS

D.M.MACDONALD AND D.C.NICHOLSON

King's College Hospital, Denmark Hill, London, S.E.5

Accepted for publication 14 November 1975

SUMMARY

The terminal stages of erythropoietic protoporphyria are recorded. The observations are related to the site of the fundamental lesion and the nature of the biochemical defect. The possibly ominous prognosis in this usually mild condition is emphasized.

Apart from congenital porphyria, the porphyrias do not usually confer severe cutaneous lesions. These diseases present to dermatologists because of moderate photosensitivity and are not usually regarded as a risk to life. Dangerous central nervous system involvement may occur, however, in acute intermittent, variegate and hereditary coproporphyrias, while in acquired symptomatic porphyria severe underlying liver dysfunction may occur.

Probably the most common familial photosensitizing porphyria is erythropoietic protoporphyria. Recently some deaths from severe liver involvement have been reported in this disease.

CASE HISTORY

H.C., male, aged 53 years

From earliest childhood H.C. experienced constant skin irritation, blistering and a wealing rash provoked by sunlight. Symptoms developed within 20 min of exposure and persisted for 3–4 h. Gray (1926) diagnosed the condition as 'hydroa aestivale without haematoporphyrinuria'. Until the definitive diagnosis was made in 1962, his symptoms were frequently considered to be functional; he was discharged from the Army in 1942 after 9 months' service, thereafter undergoing extensive psychotherapy.

In 1961 the faecal protoporphyrin varied from 1030 to 1210 μg/g of dry stool; the coproporphyrin excretion ranged from 105 to 127 μg/g dry stool. Urinary uroporphyrin reached 90 μg/day on one occasion; the red cell porphyrins were 3000 μg/100 ml of packed cells. Monochromator testing (Magnus *et al.*, 1959) in 1962 showed reactivity between 380 and 600 nm being maximal at 400 nm. At this time there was no abnormality of full blood count, liver-function tests or iron metabolism.

Family history

The family history is illustrated in Fig. 1.

Correspondence: Dr D.M.MacDonald, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

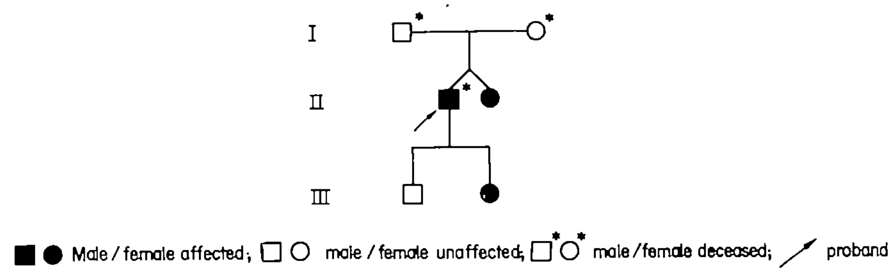

I
II
III
■ ● Male / female affected; ☐ ○ male / female unaffected; ☐* ○* male / female deceased; ↗ proband

FIGURE 1. Occurrence of clinical porphyria in the family of H.C. The patient's son and both parents are shown unaffected. The patient's twin sister and daughter have the disease.

Until 1970, liver function tests (LFTs) had shown normal results. In 1967 erythrocyte protoporphyrin levels ranged between 2200 and 3200 μg/100 ml cells while stool protophyrin and coproporphyrin levels varied respectively between 530 and 1095 μg/g dry stool, and 11–23 μg/g dry stool. Comparable levels were found in 1969 with a maximum red cell protoporphyrin of 3900 μg/100 ml cells.

In 1970 he noticed weight loss and had elevated aspartate aminotransferase levels. Liver biopsy, although now considered, was not carried out. Porphyrin levels and liver function were investigated intermittently thereafter for 3 years when he returned complaining of much-increased photosensitivity. From 1961 until his death, various therapeutic measures were used unsuccessfully. These included inosine (Gajdos, Gajdos-Torok & Mantz, 1965), administration of enterostatic resins for immobilization of intestinal porphyrins (Rimington, personal communication), treatment with β-carotene for amelioration of photosensitivity (Mathews-Roth *et al.*, 1970) and sunscreening with hydroxyacetone/lawsone cream (Runge & Fusaro, 1971). The patient claimed that occasional, self-administered ferrous gluconate reduced his photosensitivity.

In March 1974 he complained of malaise and lassitude and a new exacerbation of skin symptoms. The facial skin now presented a coarse waxy appearance having some small pitted scars, fresh vesicles and a few excoriations. The dorsa of the hands were mildly scarred. There was some icterus and gross hepatosplenomegaly. A history of abdominal pain, dark urine and pale stools of 1 month's duration was elicited.

Between 1970 and 1973 LFTs were essentially normal. However, in March 1974 his bilirubin level was estimated at 3·4 mg/100 ml with levels of aspartate amino transferase (AST) of 180 i.u./l, lactic dehydrogenase (LDH) of 225 i.u./l and alkaline phosphatase (alk. phos.) of 104 i.u./l. Up to the time of death estimation of these liver function tests reflected rapid deterioration. Shortly before death bilirubin levels reached 8·5 mg/100 ml in the blood, while the levels of AST, LDH and alk. phos. were respectively 475, 302 and 108 i.u./l.

In March and April 1974 shortly before death the red-cell protoporphyrin reached a peak of 6020 μg/100 ml cells, while porphyrin levels in the stool were 229 μg protoporphyrin per g of dry stool. Simultaneously urinary coproporphyrin levels reached 1926 μg/day with corresponding uroporphyrin level of 668 μg/day.

Other investigations (at the time of final admission to hospital)

Hb 11·4 G%, WBC 5200/mm^3, PCV 36%, platelets 78,000/mm^3, prothrombin time 5s prolonged, ESR 34 mm/h. Urea and Electrolytes were normal. Australia Antigen and Antibody −ve. α-Fetoprotein −ve. Autoantibodies −ve. *Barium swallow*, gross oesophageal varices. *Liver biopsy*, micronodular cirrhosis with cholestasis and striking deposition of pigment in hepatocytes and in the biliary canaliculi and sinusoids. The pigment exhibited characteristic protoporphyrin autofluorescence in light of 360 nm. Marked excess of a pigment resembling lipofuscin was apparent in the residue remaining from pigment extraction. *Histology* of light-exposed skin showed deposits of PAS-positive material around the dermal capillaries which showed prominent nuclei. These changes are comparable to those usually found in erythropoietic protoporphyria (Peterka, Fusaro & Goltz, 1965; Ryan, 1966; Ryan & Madill, 1968). Clinical and biochemical deterioration was rapid. As the results of LFTs and porphyrin studies became more grossly abnormal, so photosensitivity intensified. Hepatic encephalopathy developed. Treatment now included protection from light, administration of neomycin and diuretics and protein restriction. Liver transplantation, the only chance of survival, was considered but the patient died suddenly after massive haemorrhage from oesophageal varices.

DISCUSSION

The patient was a known case of erythropoietic protoporphyria (Magnus *et al.*, 1961). The abnormality of LFTs present for at least 4 years suggested that liver damage had been progressive for some time. Various abnormalities capable of affecting liver function are found in erythropoietic protoporphyria including deposition of protoporphyrin in liver cells, canaliculi and Kupffer cells, formation of gallstones, enhanced production of bilirubin and protoporphyrin in the liver and development of cirrhosis and liver failure (Cripps & Scheuer, 1965; Porter & Lowe, 1963; Redeker, Bronow & Sterling, 1963; Langhof *et al.*, 1964; Kniffen & Porter, 1969; Kniffen, 1970; Matilla & Molland, 1974). Terminal liver failure in erythropoietic protoporphyria is increasingly reported (Thompson *et al.*, 1973; Donaldson *et al.*, 1971; Barnes, Hurworth & Millar, 1968; Scott *et al.*, 1973; Schmidt & Stich, 1971). Liver histology in this case showed cholestasis with marked deposition of protoporphyrin within hepatocytes, biliary canaliculi and sinusoids. Examination under ultraviolet light showed strong red autofluorescence of this material. There was considerable bile-duct proliferation. Similar changes were reported by Thompson *et al.* (1973), Donaldson *et al.* (1971) and Barnes *et al.* (1968). Lipofuscin was also present in the liver of our patient.

The mechanism of hepatic damage in erythropoietic protoporphyria is obscure. Administration of 1,4-dihydro-2,4,6-trimethylpyridine 3,5-dicarboxylate to guinea-pigs (Granick & Urata, 1963), rats (Magnus, Roe & Bhutani, 1969), or pigs (Roe, Krook & Wilkie, 1970) causes protoporphyria, bile duct hyperplasia and liver failure but otherwise produces an imperfect analogue of erythropoietic protoporphyria. By administration of griseofulvin, Hurst & Paget (1963) and De Matteis & Rimington (1963) produced massive deposition of protoporphyrin in the livers of mice, with subsequent development of biliary cirrhosis. Rapid deterioration from progressive hepatic damage may accrue from excessive deposition of protoporphyrin in the liver with ultimate irreversible changes leading to self-perpetuating protoporphyrin retention. Gallstones, found in this condition (Cripps & Scheuer 1965; Haeger-Aronsen, 1963; Rimington *et al.*, 1967; Lynch & Miedler, 1965), are presumed to have a high protoporphyrin content, but have not been implicated in those patients who died of liver failure.

It is difficult to decide if serious liver disease is the inevitable prognosis in erythropoietic protoporphyria. Liver involvement may go undetected for a long time and, once apparent, frequently presages rapid decline. This prognosis may attend only a small number of sufferers. Variation of the severity of erythropoietic protoporphyria or hepatic complications may depend on subtle hereditary, social or environmental factors. Abatement of the cutaneous symptoms with advancing age is common (Schmidt *et al.*, 1974; Peachey & Ryan, 1967). Intrafamilial cases may vary in severity. Our patient's twin sister shows normal LFTs, only moderate red-cell protoporphyrin and little photosensitivity. A protective effect of oestrogen or a smaller hepatic component (Rimington, personal communication) may be responsible, apart from any difference in social habits, etc. Symptoms are known frequently to improve in pregnancy (Schmidt *et al.*, 1974).

Photosensitivity in erythropoietic protoporphyria is thought to be due to a high level of protoporphyrin in the skin (Magnus, 1973; Van Gog & Schothorst, 1973). Redeker & Bronow (1964) have shown that photosensitivity is specifically related to levels of plasma rather than erythrocyte protoporphyrin. The increased photosensitivity in the terminal weeks of our patient's illness may have been associated with the development of symptomatic porphyria as evidenced by greatly elevated urinary uroporphyrin. Coproporphyrin retention in the liver due to cholestasis undoubtedly caused coproporphyrinuria with the expected increase in photosensitivity.

Porphyrias are classified as hepatic or erythropoietic depending on the principal site of abnormal porphyrin metabolism (Schmid, Schwartz & Watson, 1954a, b). There remains uncertainty about the site of the porphyrin anomaly in erythropoietic protoporphyria. Evidence that some protoporphyrin is of hepatic origin (Gray *et al.*, 1964; Scholnick *et al.*, 1969, 1971; Nicholson *et al.*, 1973; Redeker & Bronow, 1964) is inconclusive. The studies of Gray *et al.* and Nicholson *et al.* were based on previous investigations in our patient. Janousek (1968, 1970) showed in griseofulvin-intoxicated mice that porphyrin was detectable in liver earlier than in erythrocytes. Matilla & Molland (1974) have recently shown ultrastructural similarity of the liver in late griseofulvin intoxication and in erythropoietic protoporphyria. They also noted that protoporphyrin accumulated in the mitochondria of the mouse hepatocyte in early griseofulvin intoxication, indicating a possible hepatic source of the pigment in both the animal model and erythropoietic protoporphyria. However, the concentration of protoporphyrin in the youngest erythrocytes suggests that their pigment is acquired immediately before release into the peripheral circulation (Clark & Nicholson, 1971). If so the disease would be truly erythropoietic as its name implies. Indeed the pattern of isotope incorporation into erythrocyte and faecal protoporphyrin has been interpreted as indicating an exclusively erythropoietic origin (Schwartz *et al.*, 1971). Liver transplantation, while offering our patient a chance of survival, would have permitted subsequent observation of any erythropoietic anomaly in isolation. Circumstances prevented this but such an important possibility should be considered in any similar clinical situation.

The nature of the biochemical defect in erythropoietic protoporphyria has not yet been fully elucidated. A block affecting incorporation of iron into the porphyrin molecule is a reasonable expectation and, if located in the bone marrow, would be compatible with the mild hypochromic and sideroblastic anaemias observed (Schmidt & Stich, 1971; Cripps & Scheuer, 1975; Donaldson *et al.*, 1971; Scott *et al.*, 1973). Raised erythrocyte protoporphyrin levels can be induced by artificial impairment of haem synthesis (Schmid *et al.*, 1954a, b) and are seen in lead poisoning and sideroblastic anaemia (Goldberg, 1968; Dacie *et al.*, 1959; Scott *et al.*, 1973). Probably an impairment of an enzyme system such as ferrochelatase is involved. This would account for the elevated erythrocyte protoporphyrin, secondary increase in 5-ALA synthetase (Miyagi, 1967; Schmidt & Stich, 1971; Cripps & McEachern, 1971) due to impaired repression consequent on lack of haem, and the observed anaemias. Whether or not the anomaly is erythropoietic exclusively, or hepatic in part, could be decided by comparative studies of the enzymology of liver and bone-marrow biopsies. The former have been made by Bottomley (1968). Similar study of bone marrow is at present complicated by the difficulty of quantifying the amounts of erythroid tissue in the biopsy material. Bottomley, Tanaka & Everett (1974), however, found a 90% decrease of erythrocyte ferrochelatase in two patients with erythropoietic protoporphyria, which strongly suggests that the erythropoietic element is of prime significance.

The therapy afforded to our patient has been described. As for all inherited diseases there are no curative regimes for the treatment of porphyrias. Recently Watson *et al.* (1974 a, b) have exploited the possibility of controlling excess porphyrin production by restoring the repression of 5-ALA synthetase with intravenous administration of haem. This proved moderately effective for acute intermittent porphyria and congenital porphyria but we were unable to apply this to our patient though it remains a therapeutic possibility for future cases.

REFERENCES

Barnes, H.D., Hurworth, E. & Millar, J.H.D. (1968) Erythropoietic porphyrin hepatitis. *Journal of Clinical Pathology*, **21**, 157.

Bottomley, S.S. (1968) Characterisation and measurement of haem synthetase in normal human bone marrow. *Blood*, **21**, 314.

Bottomley, S.S., Tanaka, M. & Everett, M.A. (1974) Diminished erythroid ferrochelatase activity in erythropoietic protoporphyria. *Clinical Research*, **22**, 384A.

Clark, K.G. & Nicholson, D.C. (1971) Erythrocyte protoporphyrin and iron uptake in erythropoietic protoporphyria. *Clinical Science*, **41**, 363.

Cripps, D.J. & McEachern, W.N. (1971) Hepatic and erythropoietic protoporphyria, δ-amino laevulinic acid synthetase, fluorescence and microfluorospectrophotometric study. *Archives of Pathology*, **91**, 497.

Cripps, D.J. & Scheuer, P.J. (1965) Hepatobiliary changes in erythropoietic protoporphyria. *Archives of Pathology*, **80**, 500.

Dacie, J.V., Smith, M.D., White, J.C. & Millin, D.L. (1959) Refractory normoblastic anaemia: a clinical and haematological study of seven cases. *British Journal of Haematology*, **5**, 56.

De Matteis, F. & Rimington, C. (1963) Disturbance of porphyrin metabolism caused by griseofulvin in mice. *British Journal of Dermatology*, **75**, 91.

Donaldson, E.M., McCall, A.J., Magnus, I.A., Simpson, J.R., Caldwell, R.A. & Hargreaves, T. (1971) Erythropoietic protoporphyria: two deaths from hepatic cirrhosis. *British Journal of Dermatology*, **84**, 14.

Gajdos, M., Gajdos-Torok, M. & Mantz, I.M. (1965) Protoporphyria erythropoietique. Traitement par l'inosine. *La Presse Medicale*, **73**, 119.

Goldberg, A. (1968) Lead poisoning as a disorder of haem synthesis. *Seminars in Haematology*, **5**, 424.

Granick, S. & Urata, G. (1963) Increase in activity of δ-amino levulinic acid synthetase in liver mitochondria induced by feeding of 3,5 dicarbethoxy 1,4-dihydrocollidine. *Journal of Biological Chemistry*, **238**, 821.

Gray, C.H., Kulczycka, A., Nicholson, D.C., Magnus, I.A. & Rimington, C. (1964) Isotope studies on a case of erythropoietic protoporphyria. *Clinical Science*, **26**, 7.

Haeger Aronsen, B. (1963) Erythropoietic protoporphyria. A new type of inborn error of metabolism. *American Journal of Medicine*, **35**, 450.

Hurst, E.W. & Paget, G.E. (1963) Protoporphyrin cirrhosis and hepatoma in the livers of mice given griseofulvin. *British Journal of Dermatology*, **75**, 105.

Janousek, V. (1968) Model Erytropoeticke Protoporfyrie-Zmeny V casnem Obdobi experimentalniho onemocneni. *Ceskoslovenska fysiologie*, **17**, 39.

Janousek, V. (1970) The early changes in experimental porphyria of mice induced by griseofulvin. *Transactions of St John's Hospital Dermatological Society*, **56**, 14.

Kniffen, J.C. & Porter, F.S. (1969) Intrahepatic porphyrastasis in erythropoietic protoporphyria. *Clinical Research*, **17**, 26.

Kniffen, J.C. (1970) Protoporphyrin removal in intrahepatic porphyrastasis. *Gastroenterology*, **58**, 1027.

Langhof, H., Heilmeyer, L., Clotten, R. & Rietschel, L. (1964) Die erythropoietische Protoporphyrie: protoporphyrinamische Lichtdermatose. *Deutsche Medizinische Wochenschrift*, **89**, 1289.

Lynch, P.J. & Miedler, L.J. (1965) Erythropoietic protoporphyria: Report of a family and a clinical review. *Archives of Dermatology*, **92**, 351.

Magnus, I.A. (1973) Porphyria and photosensitivity. *The Scientific Basis of Medicine* (Ed. by I.Gilliland and J. Francis), p. 273. Athlone Press, London.

Magnus, I.A., Jarrett, A., Prankerd, T.A.J. & Rimington, C. (1961) Erythropoietic protoporphyria. A new porphyria syndrome with solar urticaria due to protoporphyrinaemia. *Lancet*, **ii**, 448.

Magnus, I.A., Porter, A.D., McCree, K.J., Moreland, J.D. & Wright, W.D. (1959) A monochromator. An apparatus for the investigation of the responses of the skin to ultraviolet, visible and near infra red radiation. *British Journal of Dermatology*, **71**, 261.

Magnus, I.A., Roe, D.A. & Bhutani, L.K. (1969) Factors affecting the induction of porphyria in the laboratory rat. Biochemical and photobiological studies using 1:4 dihydro 2,4,6 trimethylpyridine 3,5 dicarboxylate (DOC) as a porphyrinogenic agent. *Journal of Investigative Dermatology*, **53**, 400.

Mathews-Roth, M.M., Pathak, M.A., Fitzpatrick, T.B., Harber, L.C. & Kass, E.H. (1970) Carotene as a protective agent in erythropoietic protoporphyria. *New England Journal of Medicine*, **282**, 1231.

Matilla, A. & Molland, E.A. (1974) A light and electron microscope study of the liver in a case of erythrohepatic protoporphyria and in griseofulvin induced porphyria in mice. *Journal of Clinical Pathology*, **27**, 698.

MIYAGI, K. (1967) The liver ALA synthetase activity in erythropoietic protoporphyria by means of the new micro method. *Journal of Kyushu Haematological Society*, **17**, 397.

NICHOLSON, D.C., COWGER, M.L., KALIVAS, J., THOMPSON, R.P.H. & GRAY, C.H. (1973) Isotopic studies of the erythropoietic and hepatic components of congenital porphyria and erythropoietic protoporphyria. *Clinical Science*, **44**, 135.

PEACHEY, R.D.G. & RYAN, E.A. (1967) Erythropoietic Protoporphyria (two cases—brother and sister). *Proceedings of the Royal Society of Medicine*, **60**, 497.

PETERKA, E.S., FUSARO, R.M. & GOLTZ, R.W. (1965) Erythropoietic protoporphyria. II. Histological and histochemical studies of cutaneous lesions. *Archives of Dermatology*, **92**, 357.

PORTER, F.S. & LOWE, B.A. (1963) Congenital erythropoietic protoporphyria. I. Case reports, clinical studies and porphyrin analyses in two brothers. *Blood*, **22**, 521.

REDEKER, A.G., BRONOW, R.S. & STERLING, R.E. (1963) Erythropoietic protoporphyria. *South African Journal of Laboratory and Clinical Medicine*, **9**, 235.

REDEKER, A.G. & BRONOW, R.S. (1964) Erythropoietic protoporphyria presenting as hydroa aestivale. *Archives of Dermatology*, **98**, 104.

RIMINGTON, C., MAGNUS, I.A., RYAN, E.A. & CRIPPS, D.J. (1967) Porphyria and photosensitivity. *The Quarterly Journal of Medicine*, **36**, 29.

ROE, D.A., KROOK, L. & WILKIE, B.N. (1970) Hepatic protoporphyria in weanling pigs. *Journal of Investigative Dermatology*, **54**, 53.

RUNGE, W.J. & FUSARO, R.M. (1971) Erythropoietic protoporphyria. VI. The synergistic effect of ultraviolet light and infra red radiation and protection against these wavelengths. *Acta dermato-venereologica*, **51**, 55.

RYAN, E. (1966) Histochemistry of the skin in erythropoietic protoporphyria. *British Journal of Dermatology*, **78**, 501.

RYAN, E.A. & MADILL, G.T. (1968) Electron microscopy of the skin in erythropoietic protoporphyria. *British Journal of Dermatology*, **80**, 561.

SCHMID, R., SCHWARTZ, S. & WATSON, C.J. (1954a) Porphyrins in the bone marrow and circulatory erythrocytes in experimental anaemias. *Proceedings of the Society for Experimental Biology and Medicine*, **75**, 705.

SCHMID, R., SCHWARTZ, S. & WATSON, C.J. (1954b) Porphyrin content of bone marrow and liver in the various forms of porphyria. *Archives of Internal Medicine*, **93**, 167.

SCHMIDT, H., SNITKER, G., THOMSEN, K. & LINTRUP, J. (1974) Erythropoietic protoporphyria. *Archives of Dermatology*, **110**, 58.

SCHMIDT, D. & STICH, W. (1971) Erythropoietische Protoporphyrie mit Porphyrinurie. Untersuchungen zur frage der Leberbeteiligung bei erythropoietischer Protoporphyrie. *Blut*, **22**, 202.

SCHOLNICK, P., MARVAR, H. & SCHMID, R. (1969) Erythropoietic protoporphyria—a misnomer. *Clinical Research*, **17**, 278.

SCHOLNICK, P., MARVAR, H.S. & SCHMID, R. (1971) Erythropoietic protoporphyria: evidence for multiple sites of excess protoporphyrin formation. *Journal of Clinical Investigation*, **50**, 203.

SCHWARTZ, S., JOHNSON, J.A., STEPHENSON, B.D., ANDERSON, A.S., EDMONSON, P.R. & FUSARO, R.M. (1971) Erythropoietic defects in protoporphyria: a study of factors involved in labelling of porphyrins and bile pigments from ALA-3H and glycine-14C. *Journal of Laboratory and Clinical Medicine*, **78**, 411.

SCOTT, A.J., ANSFORD, A.J., WEBSTER, B.H. & STRINGER, H.C.W. (1973) Erythropoietic protoporphyria with features of a sideroblastic anaemia terminating in liver failure. *American Journal of Medicine*, **54**, 251.

THOMPSON, R.P.H., MOLLAND, E.A., NICHOLSON, D.C. & GRAY, C.H. (1973) Erythropoietic protoporphyria and cirrhosis in sisters. *Gut*, **14**, 934.

VAN GOG, H. & SCHOTHORST, A.A. (1973) Determination of very small amounts of protoporphyrin in epidermis, plasma and blister fluids. *Journal of Investigative Dermatology*, **61**, 42.

WATSON, C.J., DHAR JEELANI, G., BOSSENMAIER, I., CARDINAL, R. & PETRYKA, Z.J. (1974a) Effect of haematin in acute porphyric relapse. *Annals of Internal Medicine*, **79**, 80.

WATSON, C.J., BOSSENMAIER, I., CARDINAL, R. & PETRYKA, Z.J. (1974b) Repression by haematin of porphyrin biosynthesis in erythrocyte precursors in congenital erythropoietic porphyria. *Proceedings of the National Academy of Science*, **71**, 278.

British Journal of Dermatology (1976) **95**, 163.

HL-A antigens in patients with guttate psoriasis

R.C.WILLIAMS,* A.W.McKENZIE, J.H.ROGER, AND V.C.JOYSEY

Previously Department of Physical Anthropology, University of Cambridge, Norfolk and Norwich Hospital, Department of Applied Statistics, University of Reading, and Tissue Typing Laboratory, Addenbrooke's Hospital, Cambridge

Accepted for publication 28 November 1975

SUMMARY

Tissue typing was performed on lymphocytes of sixty-two patients with guttate psoriasis, in forty-four of whom this was the first manifestation of the disease. In 84% of cases, the guttate psoriasis was preceded by a clinical infection. A highly significant excess of antigen W17 (HLA-BW17) was found in patients when compared with a healthy population. In common with some other studies antigens HL-A 13 (HLA-B13), W15 (HLA-BW15) and W21 (HLA-BW21) were found in excess but these became non-significant after correction for the number of antigens studied. 68% of patients who had guttate psoriasis *de novo*, subsequently developed persistent plaque psoriasis.

Antigens of the major human histocompatibility system, HL-A, have been studied previously in patients with psoriasis, and have shown an excess of W17 (BW17)† and HL-A13 (B13) (Russell, Schultes & Kuban, 1972; White *et al.*, 1972; Seignalet *et al.*, 1974; Schoefinius *et al.*, 1974; Svejgaard *et al.*, 1974; Krain, Newcomer & Terasaki., 1973), an excess of W17 (BW17) (Woodrow *et al.*, 1975), and an excess of W17 (BW17) and W16 (BW16) (Krulig *et al.*, 1975), while the HL-A1/W17 (A1/BW17) haplotype was reported to be increased by Seignalet *et al.* (1974).

In general the groups of patients studied have had psoriasis of varied morphological pattern and sometimes close definition of disease type has been lacking. White *et al.* (1972) studied patients who 'had currently active disease and were known to have had psoriasis for no less than 1 year'. Seignalet *et al.* (1974) studied a group who 'had typical lesions of psoriasis', while 16/44 of those reported by Russell *et al.* (1972) 'had apparent streptococcal flare ups' of psoriasis. In the 104 psoriatics investigated by Schoefinius *et al.* (1974), fifteen suffered from acute guttate psoriasis and eight of these had W17 (BW17). Svejgaard *et al.* (1974) subdivided their subjects into those with psoriasis vulgaris where there was a marked increase in the proportion of HL-A13 (B13) and W17 (BW17), and those with pustular psoriasis where there was no such increase compared to controls, and they used this distribution of HL-A antigens to separate the two entities. They found that 51/156 of their psoriasis vulgaris patients had increased anti-streptolysin titres (ASO) but that the frequencies of the various HL-A antigens in this group were similar to those in the remaining psoriatic patients.

---

* Present address: Veterans Administration Hospital, Ann Arbor, Michigan, U.S.A.

† The new nomenclature recognised by W.H.O. in 1975 is given in brackets.

The present study was carried out on patients who had guttate psoriasis. The term guttate psoriasis is used here to describe the clinical state in which a sudden exanthem of minute erythematous macules appears and evolves into the parakeratotic maculopapules of psoriasis. The guttate lesions measure 3–10 mm, are found particularly on the trunk and the proximal parts of the limbs, and affect mainly children and young adults. Characteristically an upper respiratory infection, usually streptococcal, occurs 1 or 2 weeks before the onset of the eruption (Winfield, 1916). The association of guttate psoriasis with preceding upper respiratory infection and raised antistreptolysin O titres has been well documented (Nørholm Pederson, 1952; Norlind, 1954; Härö, Patiala & Widholm, 1954; Whyte & Baughman, 1964).

Rapaport, Markowitz & McCluskey (1969) demonstrated cross reaction between streptococcal membrane proteins and rabbit and guinea-pig histocompatibility antigens. Because of the chemical similarity of all mammalian histocompatibility antigens, it seemed desirable to test a post-streptococcal condition for relationship to HL-A antigens.

Patients

The sixty-two unrelated Caucasoid patients in this study presented with guttate psoriasis during the period 1966–1973 and were examined by one of us (A.W.McKenzie at the Skin Clinic of the Norfolk and Norwich Hospital). In forty-four cases the condition was the first manifestation of psoriasis, and in eighteen cases the guttate exacerbation was superimposed on chronic plaque psoriasis. In most cases (52/62) a clear cut history of preceding clinical infection was obtained. In thirty-five cases antistreptolysin titres (ASO) were obtained at the time the patients first attended the clinic with guttate psoriasis. A modification of the method of Rantz & Randall (1945) was used and titres are expressed as International Units. An ASO titre of 200 units or more is regarded as evidence of recent streptococcal infection (Lawy, 1960). In five cases where no overt preceding infection had been recorded the ASO titre was nevertheless found to be elevated. At least 1 year after the patients had guttate psoriasis, they were either seen again or responded to a postal questionnaire asking the progress of their skin lesions.

Serological methods

Tissue typing was carried out using a microcytotoxic test on lymphocytes which had been cryogenically stored, as previously described (Joysey *et al.*, 1972).

Anti HL-A sera used were derived from the following sources: (1) National Institutes of Health (U.S.A.); (2) National Organ Matching Service, Bristol, England; (3) Locally produced antisera that had been used in International Histocompatibility Workshops. The specificities of the sera were checked in this laboratory by testing on a panel of non random cells whose antigenic specificities had been evaluated using the sera of the IV and V International Histocompatibility Workshops. With the exception of W19 and W21 (BW21) each specificity was represented by two or more sera in the initial tests. The following specificities were evaluated: HL-A1 (HLA-A1), 2 (A2), 3 (A3), 9 (A9), 10 (A10), 11 (A11), W28 (A28), W19 (—) HL-A5 (HLA-B5), 7 (B7), 8 (B8), 12 (B12), 13 (B13), W5 (BW35), W14 (B14), W15 (BW15), W17 (BW17), W18 (B18), W21 (BW21), W22 (BW22), W27 (B27).

Tests of any individual which yielded ambiguous results were repeated using an independent panel of eighty well authenticated anti-HL-A sera. Cells that were found in initial tests to give a positive reaction with the W21 (BW21) test serum were retested with 7 anti-W21 (BW21) sera. If all these gave a positive reaction antigen W21 (BW21) was assigned to the cells.

Statistical methods

The psoriasis patients were compared with a sample of healthy blood donors (Nelson, Darke &

Tovey, 1974). These form a 'normal' sample of the British population. It would have been preferable, but because of the shortage of some of the test sera it was not possible, to use the same sera on cells from a large random panel of control individuals.

The frequency of HL-A antigens of the psoriasis patients was compared with the 'normal' population using the two sided Fisher's exact test for 2×2 tables (Armitage, 1971).

Twenty-two antigens were tested for association with psoriasis, but using a 5% level of significance with this number of comparisons one would expect a 'significant result' to occur by chance alone. In order to compensate for this Walford, Smith & Waters (1971) advocate that the P-values be multiplied by the number of antigens tested.

RESULTS

The frequency of HL-A antigens of the psoriasis patients are compared with those of the normal population in Table 1. Differences in frequency between the patients and controls for the antigen W17 (BW17) are highly significant (P<0·00001) while significance at the 5% level was shown by HL-A1 (HLA-A1) (P = 0·02736), HL-A13 (HLA-B13) (P = 0·01536), W15 (BW15) (P = 0·02649) and W21 (BW21) (P = 0·01491). Considering a significance level of less than 0·0022 (0·05×22), to allow for the large number of tests, the value for W17 (BW17) remains significant while those for HL-A1 (HLA-A1), HL-A13 (B13) and W15 (BW15) and W21 (BW21) are rendered non-significant.

TABLE 1. Distribution of HL-A antigens in the sixty-two guttate psoriasis patients compared with that expected from the data of Nelson *et al.*, (1974) on random healthy blood donors

HL-A antigen	Observed	Expected	Control population (1036)	Exact P value
HL-A1 (HLA-A1)	30	20·8	348	0·02736
HL-A2 (HLA-A2)	33	32·1	536	0·92
HL-A3 (HLA-A3)	15	15·9	265	0·94
HL-A9 (HLA-A9)	13	11·4	190	0·71
HL-A10 (HLA-A10)	4	5·2	87	0·81
HL-A11 (HLA-A11)	4	6·3	106	0·47
HL-A28 (HLA-A28)	2	2·3	38	1·00
W19	13	10·5	175	0·50
HL-A5 (HLA-B5)	3	5·2	87	0·47
HL-A7 (HLA-B7)	8	15·9	265	0·02824
HL-A8 (HLA-B8)	13	15·6	260	0·57
HL-A12 (HLA-B12)	18	15·2	254	0·51
HL-A13 (HLA-B13)	8	2·7	45	0·01536
W5 (BW35)	5	6·8	114	0·64
W10 (BW40)	5	6·9	116	0·60
W14 (B14)	3	4·3	72	0·75
W15 (BW15)	8	3·0	50	0·02649
W17 (BW17)	30	4·7	79	<0·00001
W18 (B18)	2	2·1	35	1·00
W21 (BW21)	5	1·1	18	0·01491
W22 (BW22)	1	3·3	55	0·32
W27 (B27)	4	4·6	77	1·00

DISCUSSION

A significant excess of both W17 (BW17) and HL-A13 (B13) has been reported by several other workers. However, Seignalet *et al.* (1974) and Woodrow *et al.* (1975), as in the present study, found a very significant excess of W17 (BW17), but although an excess of HL-A13 (B13) was present in Seignalet's data this was rendered non-significant after 'correction'. This suggests that there may be heterogeneity between the series, some with a large excess of HL-A13 (B13), and some with none. This is discussed by Woodrow *et al.*

Svejgaard *et al.* (1974), as well as the strong association between psoriasis and W17 (BW17) and HL-A13 (B13), also found a non-significant excess of psoriasis patients with HL-A7 (B7), W15 (BW15) and W21 (BW21). In the present study HL-A13 (B13), W21 (BW21) and W15 (BW15) were also present in non-significant excess. As some of the associations have occurred in two independent studies, even though not significant in either, it suggests that the association between psoriasis and W15 (BW15) and W21 (BW21) might be real and not simply a feature of sampling, but larger samples would be necessary to establish this.

The present investigation confirms previous findings that there is a significant excess of W17 (BW17) in psoriatic patients. However, in the present study all the patients had guttate psoriasis, 84% of whom had an observed preceding clinical upper respiratory infection, and many others had a raised ASO titre. In the sample described here 18 patients had guttate psoriasis superimposed on pre-existing plaque psoriasis. When these plaque psoriasis patients were excluded from the series, of the forty-four cases of '*de novo*' guttate psoriasis, 20/44 possessed W17 (BW17) (comparison of W17 (BW17) with control population $P<0{\cdot}00001$). The excess of W17 (BW17) therefore is found both in patients who had only guttate psoriasis as well as those with plaque psoriasis. This would suggest that W17 (BW17) in some way predisposes the bearer to guttate psoriasis as well as to plaque psoriasis. Of the forty-four cases who had guttate psoriasis *de novo*, follow up after at least a year revealed that twenty-eight (i.e. 68·3%) had developed persistent plaque psoriasis. From a clinical point of view this study indicates that post streptoccocal guttate psoriasis is a frequent precursor of chronic plaque psoriasis.

Strong association between particular HL-A antigens and some disease states is now indisputable. A number of hypotheses had been put forward and possible causes of the associations have been summarised and discussed by McDevitt & Bodmer (1974), Dausset, Degos & Hors (1974) and by various authors in *Transplantation Reviews* (1975) edited by G.Moller; however, the mechanism of the increased susceptibility of people of a particular HL-A type (e.g. W17 (BW17)) to a given disease such as psoriasis is still a matter for discussion.

REFERENCES

Armitage, P. (1971) *Statistical Methods in Medical Research.* Blackwell Scientific Publications, Oxford.

Dausset, J., Degos, L. & Hors, J. (1974) The association of the HL-A antigens with diseases. *Clinical Immunology and Immunopathology*, **3**, 127.

Härö, A.S., Patiala, R. & Widholm, O. (1954) Psoriasis and streptococcal infections. *Annals of Internal Medicine*, **43**, 216.

Joysey, V.C., Evans, D.B., Millard, P.R. & Herbertson, B.M. (1972) A serological, clinical and morphological study of eight kidney grafts in three patients. *Tissue Antigens*, **2**, 5.

Krain, L.S., Newcomer, V.D. & Terasaki, P.I. (1973) HL-A antigens in psoriasis. *New England Journal of Medicine*, **288**, 1245.

Krulig, L., Farber, E.M., Grumet, C. & Payne, R.O. (1975) Histocompatibility (HL-A) antigens in psoriasis. *Archives of Dermatology*, **111**, 857.

Lawy, H.S. (1960) Use and interpretation of antistreptolysin test. *Annals of Rheumatic Diseases*, **19**, 42.

Moller, G. (Editor) (1975) HL-A and disease. *Transplantation Reviews*, **22**.

McDevitt, H.O. & Bodmer, W.F. (1974) HL-A, immune-response genes, and disease. *Lancet*, **i**, 1269.

Nelson, S.D., Darke, C. & Tovey, G.H. (1974) HL-A frequencies in normal blood donors, kidney donors and prospective kidney recipients. *Tissue Antigens*, **4**, 361.

Nørholm-Pederson, A. (1952) Infections and psoriasis. *Acta dermato-venereologica*, **32**, 159.

Norrlind, R. (1954) The significance of infections in the origination of psoriasis. *Acta Rheumatologica Scandinavica*, **1**, 135.

Rapaport, F.T., Markowitz, A.S. & McCluskey, R.T. (1969) The bacterial induction of homograft sensitivity. III. Effects of group A streptococcal membrane antisera. *Journal of Experimental Medicine*, **129**, 623.

Rantz, L.A. & Randall, E. (1945) Modification of technique for determination of antistreptolysin titre. *Proceedings of the Society for Experimental Biology and Medicine*, **59**, 22.

Russell, T.J., Schultes, L.M. & Kuban, D.J. (1972) Histocompatibility (HL-A) antigens associated with psoriasis. *New England Journal of Medicine*, **287**, 738.

Schoefinius, H.H., Braun-Falco, O., Scholz, S., Steinbauer-Rosenthal, I., Wank, R. & Albert, E.D. (1974) HL-A antigens and psoriasis. *New England Journal of Medicine*, **291**, 51.

Seignalet, J., Clet, J., Guilhou, J.J., Duntze, F., Meybadier, J. & Robinet-Levy, M. (1974) HL-A antigens and some immunological parameters in psoriasis. *Tissue Antigens*, **4**, 59.

Svejgaard, A., Staub Nielsen, L., Svejgaard, E., Kissmeyer Nielsen, F., Hjortshøj, A. & Zachariae, H. (1974) HL-A in psoriasis vulgaris and in pustular psoriasis—population and family studies. *British Journal of Dermatology*, **91**, 145.

Walford, R.L., Smith, G.S. & Waters, H. (1971) Histocompatibility systems and disease states with particular reference to cancer. *Transplant Reviews*, **7**, 78.

White, S.H., Newcomer, V.D., Mickey, M.R. & Terasaki, P.I. (1972) Disturbance of HL-A antigen frequency in psoriasis. *New England Journal of Medicine*, **287**, 740.

Whyte, J.H. & Baughman, R.D. (1964) Acute guttate psoriasis and streptococcal infection. *Archives of Dermatology*, **89**, 350.

Winfield, J.M. (1916) Psoriasis as sequel to acute inflammations of the tonsils. Clinical note. *Journal of Cutaneous Disease*, **34**, 441.

Woodrow, J.C., Dave, V.K., Usher, N. & Anderson, J. (1975) The HL-A system and psoriasis. *British Journal of Dermatology*, **92**, 427.

British Journal of Dermatology (1976) **95**, 169.

HL-A antigens in lichen planus

N.J.LOWE, A.G.CUDWORTH AND J.C.WOODROW

Department of Dermatology, Liverpool Royal Infirmary,
and Department of Medicine, University of Liverpool

Accepted for publication 14 October 1975

SUMMARY

A study of fifty-seven patients with lichen planus showed a significant increase in the frequency of HL-A 3 (54% compared with 29·7% in a control population). It is likely that there is a gene in the HL-A chromosomal region increasing the susceptibility to lichen planus. No correlation was found between HL-A 3 and age of onset, sex, and presence or absence of mucosal lesions.

The aetiology of lichen planus remains obscure. Evidence for the possible existence of genetic susceptibility is provided by the repeated reports of familial aggregation of the disease (Saffron, 1940; Hellier, 1950). Infective agents such as bacteria or viruses have been considered to be of aetiological importance (Brody, 1965; Swanbeck & Thyresson, 1964), and more recently there has been evidence to suggest that immunological mechanisms may also be involved (Sarkany & Gaylarde, 1972; Baart de la Faille-Kuyper & Baart de la Faille, 1974; Stankler, 1975).

Genes in the HL-A chromosomal region have been recently demonstrated to determine increased susceptibility to a considerable number of diseases (Ryder, Nielsen & Svejgaard, 1974), and the aim of this study has been to seek evidence for the existence of such genes in patients with lichen planus.

PATIENTS AND METHODS

Fifty-seven unrelated patients with established lichen planus were studied. The following features were documented; age of onset, duration of lesions and whether the lichen planus was cutaneous, mucosal or both. HL-A typing was performed using a modification of a two-stage lymphocytotoxicity method (Terasaki & McLelland, 1964). Control blood samples were obtained from blood donors residing in the same geographical area.

RESULTS

The frequencies of the HL-A antigens in the fifty-seven patients with lichen planus and 300 control subjects are shown in Table 1. The noteworthy differences are the apparent increases in the frequencies respectively of HL-A 3 and HL-A 5 in the patients as compared with controls; thirty-one of the patients (54·5%) were HL-A 3 positive as compared with eighty-nine of the controls (29·7%).

TABLE 1. Frequency of HL-A phenotypes in patients with lichen planus and controls

Antigen	Lichen planus (n = 57)	Controls (n = 300)
1st segregant series		
HL-A 1	19 (33·3)*	102 (34·0)
HL-A 2	19 (33·3)	142 (47·3)
HL-A 3	31 (54·5)	89 (29·7)
HL-A 9	9 (15·8)	60 (20·0)
HL-A 10	7 (12·3)	22 (7·3)
HL-A 11	10 (17·6)	40 (13·3)
HL-A 28	1 (1·8)	14 (5·6)
2nd segregant series		
HL-A 5	11 (19·3)	27 (9·0)
HL-A 7	19 (33·3)	94 (31·3)
HL-A 8	17 (29·8)	93 (31·0)
HL-A 12	18 (31·6)	85 (28·3)
HL-A 13	— —	17 (5·7)
HL-A 14	8 (14·0)	22 (7·3)
HL-A 17	3 (5·3)	21 (7·0)
HL-A 27	4 (7·0)	25 (8·3)
W 5	7 (12·3)	38 (12·7)
W 10	3 (5·3)	33 (11·0)
W 15	6 (10·5)	30 (10·0)
W 18	5 (8·8)	13 (4·3)
W 22	1 (1·8)	16 (5·3)

* Percentage in parentheses.

This is a significant increase in the frequency of HL-A 3; χ^2 (uncorrected) = 13·11 and P (Fisher's exact P×2) = 0·0007. The P value remains significant when the number of specificities tested for is also taken into account (i.e. 20; P = 0·014). In respect of HL-A 5, eleven of the patients (19·3%) were positive compared with twenty-six (9·0%) of the controls. The χ^2 for the difference observed = 5·34, P = 0·048. In this instance when a correction is made for the number of antigens tested, P = >0·9.

Two of the patients, both HL-A 3 positive, had affected mothers who were also HL-A 3 positive. The data were analysed in relation to the frequency of HL-A 3 and HL-A 5 and age of onset of the skin lesion, sex and presence or absence of mucosal lesions. No significant correlations were found.

DISCUSSION

The present study provides evidence that the risk of developing lichen planus is significantly increased in individuals who are HL-A 3 positive. There is weaker evidence that there may be some increased risk for HL-A 5 positive individuals. The possible mechanisms operating to produce positive associations between a disease and the HL-A system have been reviewed (McDevitt & Bodmer, 1974). The present evidence supports the concept of a gene situated in the HL-A chromosomal region increasing the susceptibility to lichen planus. It is likely, however, that such a gene only produces its effect in combination with environmental factors. Evidence obtained from studying genes in the homologous H2 region of the mouse has led to the formulation of hypotheses concerning the possible modes of action of genes in the HL-A region. In some instances at least they may act by affecting susceptibility to viruses and other infective agents, or by modifying the immune response to such agents.

The present findings suggest that further studies of the association between HL-A antigens and lichen planus should be conducted in other populations. If the association with the HL-A system is confirmed, a valuable clue to the possible aetiological mechanisms involved in the disease will have been demonstrated particularly when more is known of the mode of action of HL-A linked genes.

ACKNOWLEDGMENTS

We are grateful to our colleagues who allowed us to study their patients. Sera for typing were generously supplied by the National Tissue Typing Reference Laboratory, Bristol and by the Transplantation and Immunology Branch, N.I.H., Bethesda.

REFERENCES

BAART DE LA FAILLE-KUYPER, E.H. & BAART DE LA FAILLE, H. (1974) An immunofluorescence study of lichen planus. *British Journal of Dermatology*, **90**, 365.

BRODY, I. (1965) Electron-microscopic demonstration of bacteria in the skin of patients with lichen planus. *Nature (London)*, **207**, 96.

HELLIER, F.F. (1950) Familial lichen planus. Is it significant evidence of infectivity? *British Journal of Dermatology*, **62**, 446.

MCDEVITT, H.O. & BODMER, W.F. (1974) HL-A Immune response genes and diseases. *Lancet*, **i**, 1269.

RYDER, L.P., NIELSEN, L.S. & SVEJGAARD, A. (1974) Associations between HL-A histocompatibility antigens and non-malignant diseases. *Humangenetik*, **25**, 251.

SAFFRON, M.H. (1940) Familial lichen planus—a report of four cases of lichen planus in one family. *Archives of Dermatology and Syphilology*, **42**, 653.

SARKANY, I. & GAYLARDE, P.M. (1972) The pathogenesis of lichen planus. *British Journal of Dermatology*, **87**, 81.

STANKLER, L. (1975) Deficiency of circulating IgA and IgM in adult patients with lichen planus. *British Journal of Dermatology*, **93**, 25.

SWANBECK, G. & THYRESSON, N. (1964) Electron microscopy of intranuclear particles in lichen ruber planus: a preliminary report. *Acta dermato-venereologica*, **44**, 105.

TERASAKI, P. I. & MCLELLAND, J.D. (1964) Microdroplet assay of human serum cytotoxins. *Nature*, **204**, 998.

British Journal of Dermatology (1976) **95**, 173.

HL-A antigens and vitiligo*

G.RETORNAZ, H.BETUEL,† J.P.ORTONNE, AND J.THIVOLET

Department of Dermatology, Pavillon R, Hôpital E. Herriot, 69374 Lyon, Cedex 2, and
† Tissue-typing laboratory of the Blood-Grouping Department, 01 Beynost, France.

Accepted for publication 2 December 1975

SUMMARY

HL-A antigens were found with similar frequency in ninety vitiligo patients and in 341 healthy controls. However there was a significant correlation between HL-A 13 and vitiligo with antithyroid antibodies. This supports the concept that vitiligo is a syndrome rather than a disease.

Up to now, no specific or constant biological marker is characteristic of vitiligo. As striking relationships have been found between HL-A antigens and various diseases, the present study was undertaken to look for an association between vitiligo and the presence or absence of these antigens and to detect any possible correlation between them and aetiological, clinical or biological features.

MATERIAL AND METHODS

Ninety unrelated vitiligo patients (56 women and 34 men) of caucasoid origin were studied between October 1974 and April 1975. Diagnosis was based on clinical signs.

For HL-A typing, a two-stage technique of microlymphocytotoxicity was used according to Mittal (1968) with slight modifications (Betuel *et al.*, 1975). Ten antigens of the first locus were screened for: HL-A 1, 2, 3, 9, 10, 11, W 28, W 29, W 30, W 32, and fifteen antigens of the second locus: HL-A 5, 7, 8, 12, 13, 14, 17, 27, W5, 10, 15, 16, 18, 21, 22. At least two different antisera were used to define each antigen. The control panel consisted of 341 healthy blood donors from Lyon whose HL-A phenotypes had been clearly defined.

To estimate the degree of association between an HL-A antigen frequency in the patients and in the control group, the relative incidence ratio of Woolf was used. The relative risk $x = \frac{\text{hp}\,(1-\text{hc})}{\text{hc}\,(1-\text{hp})}$ is calculated from the antigen frequencies as found in patients (p) and in controls (c). Chi-square (χ^2) was calculated according to Woolf's method, the *P* value was multiplied by the number of HL-A antigens tested. A difference at the 5% level was considered as significant (*P* corrected).

---

* Parts of this paper are constituents of the thesis of Mrs Retornaz-Picchiottino: 'Les Groupes HL-A en Dermatologie. Revue bibliographique. Etude personnelle dans 90 vitiligos et 14 sclerodermies'. Lyon 1975 no. 305.

RESULTS

The comparison between the ninety vitiligo patients and 341 controls does not show any significant difference in HL-A antigen frequency. The same findings apply to the different subsets arranged according to sex, extent of skin lesions, involvement of the pigmentary system of hair follicles, and provocation by an external cause.

Interesting results were obtained when age at onset was considered. When the group of patients, in whom the onset of vitiligo was before 25 years, was compared to the controls, a high incidence of HL-A 12 was apparent. The high chi-square gives a corrected *P* value just short of significant. So, no HL-A type is a valid diagnostic and prognostic clue.

Twenty-three related cases of vitiligo (sisters, parents or grandparents) did not differ in their antigen frequency from cases with no family history of the disease. In a three-generation family, four of the five members afflicted with vitiligo were HL-A typed. No haplotype appeared to be correlated with the skin disease.

Four of the ninety patients had thyroid disease, and sixteen patients had one relative with thyroid

TABLE 1. HL-A antigens in vitiligo patients with and without antithyroid antibodies

HL-A antigen	Antithyroid antibodies + (n = 13)		Antithyroid antibodies − (n = 44)		Relative risk
	No.	%	No.	%	
HL-A 1	2	15·4	7	15·9	0·96
2	9	69·2	28	63·6	1·28
3	1	7·7	13	29·5	0·19
9	2	15·4	8	18·2	0·81
10	1	7·7	4	9·1	0·83
11	1	7·7	1	2·3	3·58
W 28	1	7·7	5	11·4	0·64
29	1	7·7	2	4·5	1·75
30	2	15·4	7	15·9	0·96
32	1	7·7	1	2·3	3·58
HL-A 5	1	7·7	9	20·8	0·32
7	0	0	8	18·2	0·15
8	2	15·4	4	9·1	1·81
12	6	46·1	15	34·1	1·65
13	4	30·8	1	2·27	19·1
14	3	23·1	3	6·8	4·09
17	0	0	2	4·5	0·62
27	1	7·7	3	6·8	1·13
W 5	3	23·1	6	13·6	1·89
10	1	7·7	2	4·5	1·75
15	0	0	2	4·5	0·62
16	0	0	1	2·3	1·07
18	2	15·4	3	6·8	2·48
21	0	0	4	9·1	0·33
22	2	15·4	4	9·1	1·81

disorder. In this group of twenty patients the HL-A distribution pattern was similar to that of the other vitiligo patients and the healthy controls.

Thirteen vitiligo patients with antithyroid antibodies (detected by a double layer immunofluorescence technique) (Guinet *et al.*, 1967) were compared to forty-four patients without these antibodies and to the 341 controls (Table 1). An increased frequency of HL-A 13 was apparent: four patients (30·8%) had this antigen as compared to fourteen controls (4·1%). The relative risk is 10·3; the high chi-square 12·57 gives a *P* value <0·001 significant after correction (<0·025). Relative risks in the patients with and without antithyroid antibodies are not significant, even for HL-A 13.

DISCUSSION

The frequency of antithyroid antibodies in vitiligo has been diversely reported (Bor *et al.*, 1969; Brostoff, 1969; Cunliffe *et al.*, 1968; Grupper *et al.*, 1970; Ortonne, 1974; Perrot *et al.*, 1973; Woolfson *et al.*, 1975). The exact implication of their presence is unknown. The association between vitiligo and thyroid disease is unrelated to any HL-A antigen, particularly HL-A 13. HL-A 13 is not associated as a rule with antithyroid antibodies (Grumet *et al.*, 1974). HL-A 13 has so far not been implicated in any autoimmune disorder (Dausset, 1975).

The correlation between HL-A 13 and vitiligo with autoimmunity supports the concept that vitiligo is more a syndrome than a disease. Vitiligo without biological criteria of autoimmunity can be distinguished from vitiligo with antithyroid antibodies.

REFERENCES

Betuel, H., Camoun, M., Day, N.E., Ellouz, R. & The, G. (1975) The relationship between nasopharyngeal carcinoma and the HL-A system among Tunisians. *International Journal of Cancer*, **16**, 249.

Bor, S., Feiwel, M. & Chanarin, I. (1969) Vitiligo and its aetiological relationship to organ-specific autoimmune disease. *British Journal of Dermatology*, **81**, 83.

Brostoff, J. (1969) Autoantibodies in patients with vitiligo. *Lancet*, **ii**, 177.

Cunliffe, W.J., Hall, R., Newell, D.J. & Stevenson, C.J. (1968) Vitiligo, thyroid disease and autoimmunity. *British Journal of Dermatology*, **80**, 135.

Dausset, J. & Hors, S. (1975) Some contributions of the HL-A complex to the genetics of human disease. *Transplantation Reviews*, **22**, 44.

Grumet, F.C., Payne, R.O., Konishi, J. & Kriss, J.P. (1974) HL-A antigens as markers for disease susceptibility and autoimmunity in Grave's disease. *The Journal of Clinical Endocrinology and Metabolism*, **39**, 1115.

Grupper, Ch., Benazeraf, C., Rousselet, G. & Eyquem, A. (1970) Bilan biologique et immunologique chez 70 malades atteints de vitiligo. *Bulletin de la Société Française de Dermatologie et Syphiligraphie*, **77**, 852.

Guinet, P., Mornex, R., Pousset, G., Monier, J.C. & Thivolet, J. (1967) Utilisation de la technique d'immunofluorescence pour la recherche des anticorps antithyroidiens. *Lyon Médical*, **50**, 1523.

Mittal, K.K., Mickey, M.R., Singal, D.P. & Terasaki, P.I. (1968) Serotyping for transplantation. XVIII. Refinement of Microdroplet lymphocyte cytotoxicity test. *Transplantation*, **6**, 913.

Ortonne, J.P. (1974) Le vitiligo: maladie ou syndrome? *Thèse médecine Lyon*, p. 243.

Perrot, H., Pousset, G., Monier, J.C., Tourniaire, J. & Thivolet, J. (1973) Vitiligo, thyréopathies et autoimmunisation. *Lyon Médical*, **230**, 325.

Woolfson, H., Finn, O.A., Mackie, R.M., McQueen, A. & McSween, R.N.M. (1975) Serum anti-tumour antibodies and auto-antibodies in vitiligo. *British Journal of Dermatology*, **92**, 395.

British Journal of Dermatology (1976) 95, 177.

Pharmacology and Treatment

Clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules for the treatment of skin infections

M.EL-ZAWAHRY, ABBAS ABDEL AZIZ, MOHSEN SOLIMAN,
S.SHOEIB AND S.S.TADROS*

Department of Dermatology and Bacteriology, Cairo University, and *The Squibb Institute for Medical Research, Cairo, Egypt

Accepted for publication 7 November 1975

SUMMARY

Fifty-five patients with impetigo, ecthyma, or folliculitis caused by epicillin-sensitive strains of *Streptococcus pyogenes* or *Staphylococcus aureus* were treated with 500 mg epicillin capsules twice daily for an average duration of 9 days. Bacteriological and clinical examinations were performed at 2-day intervals. Favourable clinical responses (excellent or good) were obtained in 95% of the patients. No side effects were observed.

Bacterial infection of the skin is one of the most common medical disorders in Egypt (Zawahry, 1963). *Staphylococcus aureus* and *Streptococcus pyogenes* are the usual organisms isolated from patients with impetigo contagiosa (Zawahry, Aziz & Soliman, 1972). In many instances the infections are too extensive for local therapy.

Systemic antibiotic therapy is recommended for widespread or complicated infections and also to avoid complications (Dillon *et al.*, 1967; Dillon, 1968). Oral administration is usually preferred, because the convenience of this mode of therapy to the patients usually results in better compliance with the suggested dosage regimen. Epicillin has been reported to have a broad spectrum of activity against most of the organisms causing skin infections with the exception of penicillinase producers (Basch, Erickson & Gadebusch, 1971; Gadebusch *et al.*, 1971; Reyes-Javier, 1971).

This study is an evaluation of the semisynthetic penicillin, epicillin (Squibb 11,302) which is chemically designated as 6-(D-2-amino-2-(1,4-cyclohexadien-1-yl)acetamido) penicillanic acid, in a twice daily oral dosage schedule for the treatment of patients with skin infections produced by susceptible organisms.

PATIENTS AND METHODS

Fifty-five patients (39 males and 16 females) were evaluated in this study; thirty-seven were suffering from impetigo, ten from ecthyma, and eight from furuncles and folliculitis.

The patients' ages ranged from 6 to 47 years with a mean age of 17·8 years for all groups; body weights ranged from 24 to 98 kg with a mean weight of 50·9 kg.

A complete medical history was obtained and a physical examination was performed prior to initiation of therapy. Patients with a past or present history of rheumatic fever, chronic dermatological problems, diabetes, neoplastic diseases, or who were receiving immunosuppressive agents or other antibiotics were not enrolled in this study. In addition, patients with a history of hypersensitivity to penicillins or cephalosporins were excluded.

Pretreatment specimens for culture were obtained from the skin lesions of each patient in order to identify the infecting organism and to determine its sensitivity to epicillin using 10 μg sensitivity discs and the Kirby–Bauer method (Bauer *et al.*, 1966). All cultures of *S. aureus* and beta-haemolytic streptococci having an inhibition zone of more than 25 and 13 mm respectively were considered sensitive. Cultures with a smaller zone size were considered negative.

Only patients with infections caused by Group A beta-haemolytic streptococci or by staphylococci that did not produce penicillinase were eligible for admission to the study. Therapy was usually started after proof of sensitivity of the micro-organism to epicillin was obtained and cases with resistant organisms were not given the test drug.

However, when treatment was initiated prior to receiving culture results, therapy was continued in patients who had an infection caused by a resistant organism but in whom a distinct clinical improvement was obvious by the time culture results were obtained. Otherwise, patients with infections caused by resistant organisms or any organism other than the two mentioned above were discontinued from therapy and were excluded from the evaluation. Twelve of the fourteen patients excluded 48 h after commencing therapy had infections caused by resistant strains of *S. aureus*, one by *S. albus*, and one by *Mima polymorpha*.

All fifty-five patients included in the efficacy analysis had follow-up cultures and sensitivity testing done 48 h, 4 days and 6 days after initiation of therapy, and 48 h after the completion of therapy. Complete healing of skin lesion precluded any further cultures. The patients were examined as frequently as possible, the minimum being on the days scheduled for microbiological procedures. Patients suffering from infections caused by *Str. pyogenes* were also seen after 30 days and cultures were performed if lesions persisted.

One epicillin capsule (500 mg) was given every 12 h, regardless of age and weight, for a minimum of 5 days and maximum of 14 days. All infections caused by beta-haemolytic streptococci were treated for a minimum of 10 days to guard against the known sequelae of such infections (McCollough *et al.*, 1951; Markowitz *et al.*, 1965). No other antibiotic, anti-infective agent, or probenecid was allowed during therapy with epicillin.

Evaluation of responses was based on all factors involved, especially the severity of infection, the first signs of improvement appeared, and the time of greatest improvement.

RESULTS

The general condition of the patients at the start of therapy was good in forty-eight and fair in seven. The infections were judged to be mild in fourteen patients, moderate in thirty-four, and severe in seven. The duration of illness prior to therapy was 10 days or less in twenty-seven patients, 11–20 days in fifteen, and more than 20 days in thirteen.

The results of pretreatment sensitivity test for cases evaluated for efficacy appear in Table 1.

The average duration of treatment was 9 days and the average total dose of epicillin received by the patients was 9 g. Average times of initial and maximum improvement of signs and symptoms were 2 and 5·5 days, respectively. The mean for each type of infection was not very different from the mean for all infections (Table 2). Table 3 shows that the overall response to therapy was judged to be Excellent in twenty-eight patients (51%), Good in twenty-four (44%), Fair in two (3%), and Poor in one patient (2%).

Cultures obtained 48 h after therapy commenced, showed eradication of *S. aureus* in four (9%) and *Str. pyogenes* in twelve (60%) samples. On the 4th day of therapy, cultures were negative for *S. aureus* in twenty-nine (87%) and *Str. pyogenes* in eighteen (90%). Cultures did not show growth of *S. aureus* from forty-three samples (96%) and *Str. pyogenes* from twenty samples (100%) on the 6th day of therapy. Forty-eight hours after completing therapy, *S. aureus* and *Str. pyogenes* were completely eradicated in forty-four (98%) and twenty cultures (100%) respectively. No significant change in the sensitivity of any organism to the antibiotic was observed during therapy.

DISCUSSION

The treatment of skin infections may be difficult due to the increased resistance of infecting organisms

TABLE 1. Pretreatment sensitivity of causative organisms correlated to primary diagnosis

Diagnosis	No. of cases	No. of cases caused by the 2 organisms concurrently	Total no. organisms	*S. aureus*			*Str. pyogenes*		
				Sensitive	Resistant	Total	Sensitive	Resistant	Total
Impetigo	37	7	44	25	6	31	13	0	13
Ecthyma	10	3	13	4	2	6	7	0	7
Furunculosis & Folliculitis	8	0	8	7	1	8	0	0	0
Total	55	10	65	36 (80)*	9 (20)	45 (100)	20 (100)	0 (0)	20 (100)

* Percentages in parentheses.

TABLE 2. Duration of therapy and time to improvement

	Impetigo	Ecthyma	Folliculitis	Total
No. of cases	37	10	8	55
Improvement of signs and symptoms noted:				
First (mean) (55 patients)	2·19	1·90	2·13	2·13
Maximum (mean) (54 patients)	5·59	5·30	5·28	5·5
Mean duration of treatment (in days)	8·9	9·25	8·75	8·95

TABLE 3. Overall response to therapy

Overall response to therapy	Impetigo	Ecthyma	Folliculitis	Total
Excellent	15	8	5	28 (51)*
Good	20	2	2	24 (44)
Fair	2	0	0	2 (3)
Poor	0	0	1	1 (2)

* Percentage in parentheses.

to commonly used antibiotics. Connor (1972) found that 95% of the Staphylococci isolated from impetigo lesions were resistant to penicillin and 50% to tetracycline. When treating pyodermas in children, Golosovker (1961) noted that all staphylococci isolated (170 strains) except one were resistant to penicillin. Chloramphenicol has been described as one of the antibiotics for the treatment of resistant strains in staphylococcal infections (Kirby & Ahren, 1953) but its use is limited by its toxic side effects.

The results obtained in our study indicate an excellent or good response in 95% of the patients treated without the development of epicillin resistant strains during therapy. Forty-six of the patients included in the study fulfilled the protocol requirements devised for this study which entailed the admission of cases caused only by sensitive strains of *S. aureus* and beta-haemolytic streptococci.

Patients with a pretreatment resistant organism who did not receive epicillin were not admitted to the study. In some cases we started therapy immediately after taking the pretreatment specimen. Two days later, they presented for the first follow-up and by that time culture results were available. If the organism was sensitive, therapy was continued. If evident clinical improvement had occurred despite the organism showing *in vitro* resistance to epicillin, we decided to let those cases continue assuming that the organism was sensitive *in vivo*. None of these cases is reported in this series, four of which were caused by a sensitive *Str. pyogenes* and a resistant *S. aureus* present concurrently. Surprisingly the resistant *S. aureus* in one of these cases could not be grown in the first follow-up culture. The other five cases were caused by resistant *S. aureus* only, which was eradicated on the second follow-up culture (three cases), on the fifth (one case) and never eradicated (one case).

All other cases with resistant organisms were discontinued from therapy after 48 h and were not included in the evaluation for efficacy (twelve cases). No untoward effect, either local or general, was observed in this study. The apparent prolongation of treatment time was due to the recommended long course of treatment indicated for streptococcal infections.

Thus, 500 mg capsules of epicillin given twice daily can be recommended for the treatment of skin infections caused by susceptible organisms, due to its efficacy, safety, and convenience of administration.

REFERENCES

BASCH, H., ERICKSON, R. & GADEBUSCH, H. (1971) Epicillin: *In vitro* laboratory studies. *Infection and Immunity*, 4, 50.

BAUER, A.W., KIRBY, W.M.M., SHERRIS J.C. & TURCK M. (1966) Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 45, 493.

CONNOR, B.L. (1972) Impetigo contagiosa in the United Kingdom. *British Journal of Dermatology*, Suppl. 8, 48.

DILLON, H.C., JR. (1968) Impetigo contagiosa: suppurative and non-suppurative complications. *American Journal of Diseases of Children*, 115, 530.

DILLON, H.C. JR., MOODY, M.D., MAXTED, W.R. & PARKER, M.T. (1967) The epidemiology of impetigo and acute glomerulonephritis. Results of serological typing of group A streptococci. *American Journal of Epidemiology*, 86, 710.

GADEBUSCH, H., MIRAGLIA, G., PANSY, F. & RENZ, K. (1971) Epicillin: experimental chemotherapy, pharmacodynamics, and susceptibility testing. *Infection and Immunity*, 4, 50.

GOLOSOVKER, S. YA. (1961) Pyoderma in children (Russian) Trudy V Vses. Sezda Derm.-Vener. (Leningrad), 264 [Abstract: (1963) Exerpta Medica. Dermat. and Vener., 17, sect. 13, 22.]

KIRBY, W.M.M. & AHERN, J.J. (1953) Changing pattern of resistance of staphylococci to antibiotics. *Antibiotics and Chemotherapy*. Vol. III, No. 8, p. 831.

MARKOWITZ, M., BRUTON, M.D., KUTTNER, A.G. & CLUFF, L.E. (1965) The bacteriologic findings, streptococcal immune response and renal complications in children with impetigo. *Pediatrics*, 35, 393.

MCCULLOUGH, G.C., COFFEE, J.Y., TRICE, P.A., JR., STONE, J.J. & CRANDALL, H.L. (1951) Acute glomerulonephritis. Impetigo as an aetiological factor; a review of 124 cases at Lloyd Noland Hospital, Fairfield, Ala. *Journal of Pediatrics*, 38, 346.

REYES, JAVIER, P.D. (1971) Epicillin (Dexacillin®) in the treatment of acute superficial and soft tissue infections. *Current Therapeutic Research*, 13, 302.

ZAWAHRY, M. EL. (1963) *Skin Diseases in Arabian Countries*, Vol. 1. Published by the Author.

ZAWAHRY, M. EL., AZIZ, A.A. & SOLIMAN, M. (1972) The aetiology of impetigo contagiosa. *British Journal of Dermatology*, 87, 420.

British Journal of Dermatology (1976) **95**, 181.

Erythema multiforme in children

RESPONSE TO TREATMENT WITH SYSTEMIC CORTICOSTEROIDS

JAMES E.RASMUSSEN

Departments of Dermatology and Pediatrics, State University of New York at Buffalo,
Children's Hospital of Buffalo, New York, U.S.A.

Accepted for publication 7 November 1975

SUMMARY

It is generally accepted that the correct treatment for patients with severe erythema multiforme is systemic corticosteroids. This paper is a review of thirty-two paediatric patients with severe erythema multiforme (Stevens–Johnson syndrome) who were treated with either large doses of systemic corticosteroids or supportive care only. Those patients treated with steroids did not recover sooner than those treated in other fashions and the steroid treated group had a significant incidence of medical complications. This retrospective study proves nothing but it does suggest that treatment of patients with the Stevens–Johnson syndrome with systemic corticosteroids may be associated with significant side effects and prolonged recovery.

Erythema multiforme (EM) is a distinct clinical entity whose aetiology is diverse and usually remains enigmatic. Inflamed cutaneous lesions—papular, iris or target—are sharply marginated, relatively non-pruritic and located primarily on the extremities. The inciting agent, whether mediated by a primary reaction acting directly on the skin and mucous membranes or through secondary chemical mediators, produces a spectrum of responses from erythema to haemorrhage, bullae and necrosis. Any theory of pathogenesis must also explain the febrile reaction that almost always accompanies the exanthem and enanthem.

A wide variety of agents have been reported to be associated with EM: infectious organisms such as bacteria (Washington, Fowler & Guarino, 1967; Meyers, 1970), mycoplasma (Gordon & Lyell, 1970), herpes (Shelley, 1967), fungi (Medeiros *et al.*, 1966) and parasites (March, 1965); drugs such as long acting sulphonamides, oral contraceptives, dilantin (Bianchine *et al.*, 1968); chemicals such as 9-bromofluorene (DeFeo, 1966); and a variety of other diseases. A considerable number of patients with erythema multiforme have no such associations and it must be presumed that EM has a variety of causes working through a final common pathway.

The therapy of erythema multiforme (EM) has been established by convention. The clinical features of this disease and the effectiveness of systemic corticosteroids in abolishing them have been accepted by most authors (Lyell, 1971; Domonkos, 1970; Pearson, 1972; Costello & DeFeo, 1963; Hambrick, 1965; Coursin, 1966). There is, however, little published evidence that corticosteroids control the

Reprint requests to: JER, Children's Hospital of Buffalo, 219 Bryant St., Buffalo, New York 14222, U.S.A.

inflammatory component of erythema multiforme or accelerate healing. This paper is an analysis of thirty-two paediatric patients with Stevens–Johnson syndrome (SJS) which focuses on their response to treatment.

MATERIAL AND METHODS

The criteria for inclusion in this study were: admission to the Buffalo Children's Hospital; and a clinical description consistent with erythema multiforme of the SJS variety, i.e.

1. Severe inflammation and erosion of the oropharynx and either conjunctivitis, vaginitis or balanitis.
2. Erythematous papules and plaques or bullae with darker centers that slowly expand but do not migrate.

It was not necessary for a patient to have iris or target lesions to be included in this study.

RESULTS

Thirty-two patients met the criteria for SJS (Tables 1–3). The 17 males and 15 females, whose age varied from 8 months to 14 years, were admitted from 1952 to 1974. Increased incidence of admissions occurred during the autumn and winter months of October to May but the disease was clinically similar regardless of when the patients were admitted. Twenty-three of the thirty-two patients had a febrile prodrome that featured pharyngitis and conjunctivitis and five patients in this group received antibiotics for these symptoms—penicillin (two patients), sulphonamides (two patients) and lincomycin (one patients). Four other children in this series were each exposed to one drug during the prodrome of their illness—xylocaine for a dental extraction, diphenylhydantoin, butisol and a diphtheria–pertussis–tetanus vaccination.

TABLE 1. Thirty-two patients

	Steroid treated (17)	No steroids (15)
Age (mean)	6 yr, 4 months	5 yr, 5 months
Sex	8 M, 9 F	9 M, 6 F
Duration of prodrome before hospitalization (days) (mean)	4	3
Number of patients with prodrome	13	10
Exposure to drugs before hospitalization	2	3
Initial fever (mean)	39·6°C	39·8°C
Initial WBC (mean)	12,400/mm^3	14,200/mm^3
Other treatment (antibiotics)	8	5

Because of the selection of cases, extensive oral inflammation and erosion were always present and haemorrhagic labial crusts were a common accompaniment. Conjunctivitis was also frequent and severe in this group of patients (26/32) and was usually bilaterial. Erythema, haemorrhage and erosions also affected the anal (4/32) or genital areas (9/32).

Erythematous papules and bullae were a prominent component of the SJS in this group of patients. The lesions were arbitrarily called localized if confined to the extremities and generalized if they were also present on the trunk. Seventeen of the thirty-two patients had iris lesions.

Laboratory

Cold agglutinins were found in titres less than 1:8 in 11/15 patients and were never higher than 1:64 (1/15 patients). A variety of pathogenic bacteria were cultured from the oropharynx (21/32) (Table 3), but thirty-five blood cultures taken on the day of admission in seventeen patients were all sterile. Three patients subsequently developed sepsis with positive blood cultures (staphylococcus in one patient and pneumococcus in two).

Treatment

Seventeen patients received systemic corticosteroids. Corticosteroids were used on a basis of 40–80 mg/m^2 of prednisone or equivalents per day and they were always begun on the 1st or 2nd day of hospitalization. Systemic antibiotics were used in 8/17 patients in the steroid group and 5/15 in the non-steroid group. Penicillin was the most popular choice followed by ampicillin and erythromycin.

TABLE 2. Severity and extent

	Steroid treated (17)	No steroids (15)
Mouth and eyes	12	11
Rectum, vagina, penis	5	4
Papules—local	4	4
generalized	6	4
Bullae—local	3	4
generalized	1	3

TABLE 3. Pathogens isolated on initial throat culture

	Steroid treated (17)	No steroids (15)
Staphylococcus	4	3
Pneumococcus	2	4
β Haemolytic streptococcus	3	1
Mycoplasma	1	1
H. Influenzae	2	0

Recovery and complications

Nine patients had a complicated course before recovery. These patients were all in the corticosteroid-treated group. It was not possible to state with certainty when active inflammation began to subside in either the steroid- or the non-steroid-treated groups but the average length of hospitalization was significantly different. The fifteen patients in the non-steroid group were in the hospital for an average of 13 days compared to a 21 day hospitalization for those treated with steroids ($P < 0{\cdot}01$).

DISCUSSION

The severity of erythema multiforme is quite variable but there is a general advocacy of the use of systemic corticosteroids and widespread testimonial to their effectiveness. The strength of this belief is quite strong, although there are some steroid iconoclasts (Lyell, 1968; Editorial, 1972; Claxton, 1963; Blank, 1974). The difficulty in validating therapeutic effectiveness has been the almost complete lack of data. Most authors have emphasized the association between erythema multiforme and suspected causal agents and have not analyzed treatment and its relation to recovery.

In order to obtain a definable and equivalent group this study reports only paediatric patients with severe erythema multiforme. The thirty-two patients reported all had SJS. Fever and pharyngitis were the nonspecific prodromes and subsequent oral erosions and conjunctivitis the hallmarks. Clinical and epidemiological data were presented to compare the methods of treatment.

Systemic corticosteroids were used in 17/32 patients. The age, sex, length of prodrome, exposure to drugs, initial fever, extent of oral and cutaneous involvement and frequency of isolation of pathogens were equivalent in the patients in the steroid- and in the non-steroid-treatment groups (Tables 1–3). The only obvious differences were the drugs used to treat each group and their responses. It was impossible to evaluate accurately the immediate responses of the erosions and inflammatory cutaneous lesions to systemic steroids. There is a definite point in the history of each patient with erythema multiforme when the margins of the cutaneous lesions are no longer inflammatory and develop a red–blue colouration that signals the onset of spontaneous resolution. This point was not reported in enough patients to be a significant factor in analysis and other parameters were used as a measurement of response to steroids—duration of fever, length of hospitalization and frequency of complications.

Fever was definitely affected by systemic corticosteroids lasting an average of 1·8 days when these agents were used and 5·5 days when they were not. Maximum elevation was the same for both groups, averaging 39·6°C rectally for those treated with steroids and 39·8°C in the non-steroid group.

Length of hospitalization was also significantly different between the two groups—21 days for those using steroids and 13 days for those in the non-steroid group ($P < 0{\cdot}01$). The length of hospitalization was directly related to the incidence of complications, however, and if those patients with SJS and complications were excluded from the steroid treated group then the length of hospitalization was similar to those not treated with steroids (13 vs 15 days).

Nine patients had a total of ten complications, which were defined as any medical, surgical or psychiatric problem which arose after admission for SJS and which was not a component of the presenting complaint. All patients with complications were in the steroid-treated group. Infection was the most common complication, occurring in four patients. There were two cases of pneumococcal pneumonia and one of staphylococcal pneumonia all of which eventuated in bacterial sepsis with positive blood cultures. None of these organisms was present on initial throat culture. None of these patients had had concurrent treatment with antibiotics. One other patient developed widespread herpes simplex of the face and ocular conjunctivae.

Significant gastro-intestinal bleeding, necessitating transfusion, occurred in three patients, all of whom had been treated with steroids. Bloody diarrhoea was noted in two patients and the third vomited blood followed by melaena. The bleeding sites were not identified in any of the patients but the history suggested a distal site in the first two patients and a proximal location in the last. Bleeding terminated spontaneously in 4–7 days and was never massive enough to require surgical exploration.

The use of systemic steroids was associated with the onset of fits, apnoea and pseudotumour-cerebri in one patient with SJS and Cushingoid facies, oedema of the legs and pulmonary effusion in another. Another patient had a significant leukopenia (white blood cells = 1800/mm^3) for 9 days which slowly returned to normal.

All the complications occurred in those SJS patients treated with systemic steroids. In addition, the length of hospitalization for those treated with steroids (21 days) was considerably greater than the non-steroid group (13 days) but was equivalent when the patients with complications were excluded from the statistics. Neither the number of complications nor the length of hospitalization indicates that treating SJS with systemic corticosteroids confers any direct beneficial effects. Although it is impossible to say with certainty, the relatively large doses of corticosteroids used to treat these patients with the Stevens–Johnson syndrome may have contributed to the relatively high incidence of side-effects. Although it is customary to use prednisone on a 40–60 mg/m^2 or equivalent basis some authorities believe that this dose is too high.

To imply that all the side-effects in the steroid group were directly related to the drugs used would be naive. Medical and surgical complications have been noted in patients with SJS before corticosteroids were available for use. Yet to assume that steroids were used only in patients at great risk of complications would be too simple. Most steroid treated patients received these drugs because their physician uses them in all patients with the SJS and not just in the most ill patients. Some of the complications are known to occur with steroid usage (David, Grieco & Cushman, 1970)—infection, pseudotumour-cerebri and Cushingoid facies with fluid retention; for others—gastro-intestinal bleeding and leukopenia—the associations are debatable. Steroids did not, however, reduce the length of hospitalization in patients with SJS without complications.

The possible deficiencies in this type of retrospective study are serious and make it difficult to draw any valid conclusions. There are at least three major criticisms of this study. (1) Were the groups of patients treated with corticosteroids identical to those not given steroids? They were not identical but equivalent. The severity of the disease as measured by length of prodrome, initial fever, exposure to drugs and extent of lesions was similar in both groups. (2) Was there a difference in aetiologies among the groups of patients with SJS? The cause of SJS can only rarely be identified. The numbers of positive throat cultures and exposures to drugs were similar for the two groups of patients. No patient had a previous history of herpes simplex. (3) Why were some patients treated with corticosteroids and others not? The decision to use systemic corticosteroids did not seem to be dictated on the basis of the severity of the disease. All patients had severe oral erosions, most had a significant conjunctivitis and all patients were admitted to the hospital. Some physicians in this community use systemic corticosteroids for all their patients with SJS and others never use these drugs. Systemic corticosteroids have not been used in this hospital for the past 2 years, a time when four patients with severe SJS were treated on the wards without complications.

This study cannot answer the question of the efficacy of systemic corticosteroids in SJS. It does indicate, however, that their accepted therapeutic efficacy should be reviewed. This suggestion may not be too radical if one considers that the prodromal and inflammatory stages of erythema multiforme are very short. The initial erythema usually fades in 3–7 days leaving the erosions, bullae and haemorrhagic papules, and any beneficial effect might not be measurable in a disease of such short duration. When corticosteroids are used infection is one of the most common complications. Effectiveness of prophylactic antibiotics is difficult to evaluate and their use may be associated with beneficial effects or the development of infection with resistant organisms. While steroids may quieten inflammation, they should not accelerate wound healing.

REFERENCES

BIANCHINE, J.R., MACARAEG, P.V.J., JR. & LASAGNA, L., *et al.* (1968) Drugs as etiological factors in the Stevens–Johnson syndrome. *American Journal of Medicine*, 44, 390.

BLANK, H. (1974) Corticosteroids in cutaneous diseases. I. A practical guide to safe and effective use of steroidal drugs. *Modern Medicine*, 42, 18.

CLAXTON, R.C. (1963) A review of 31 cases of Stevens–Johnson syndrome. *Medical Journal of Australia*, 1, 1963.

COSTELLO, M.J. & DE FEO, C.P. (1963) Recent advances in the diagnosis and treatment of bullous dermatoses. *New York State Journal of Medicine*, 63, 3213.

COURSIN, D.B. (1966) Stevens–Johnson syndrome: nonspecific parasensitivity reaction? *Journal of the American Medical Association*, **198**, 113.

DAVID, D.S., GRIECO, M.H. & CUSHMAN, P. (1970) Adrenal glucocorticoids after 20 years. A review of their clinically relevant consequences. *Journal of Chronic Diseases*, **22**, 637.

DEFEO, C.P., JR. (1966) Erythema multiforme bullosum caused by 9-bromofluorene. *Archives of Dermatology*, **94**, 545.

DOMONKOS, A.N. (1970) *Andrew's Diseases of the Skin*, p. 142. W.B. Saunders, Philadelphia.

Editorial (1972) Erythema multiforme. *British Medical Journal*, **i**, 63.

GORDON, A.M. & LYELL, A. (1970) Mycoplasmas and their association with skin disease. *British Journal of Dermatology*, **82**, 414.

HAMBRICK, G.W., JR. (1965) The treatment of erythema multiforme, dermatitis herpetiformis and pemphigus. *Modern Treatment*, **2**, 916.

LYELL, A. (1971) *Erythema Multiforme in Dermatology and General Medicine* (Ed. by T.B.Fitzpatrick, K.A.Arndt and W.H.Clark, Jr.), p. 598. McGraw Hill, New York.

LYELL, A. (1968) Erythema multiforme: manifestations and management. *Current Medicine and Drugs*, **8**, 3.

MARCH, C.H. (1965) Erythema multiforme bullosum associated with trichomonas infection. *Archives of Dermatology*, **92**, 674.

MEDEIROS, A.A., MARTY, S.D., TOSK, E.E. & CHIN, T.D.Y. (1966) Erythema nodosum and erythema multiforme as clinical manifestations of histoplasmosis in a community outbreak. *New England Journal of Medicine*, **27**, 415.

MEYERS, W. (1970) Erythema multiforme. *Archives of Dermatology*, **101**, 707.

PEARSON, R.W. (1972) *Blistering Diseases in Pediatrics* (Ed. by H.L.Barnett and A.H.Einhorn), p. 1770. Appleton-Century-Crofts, New York.

SHELLEY, W.B. (1967) Herpes simplex virus as a cause of erythema multiforme. *Journal of the American Medical Association*, **201**, 153.

WASHINGTON, J.L., FOWLER, R.E.L. & GUARINO, G.L. (1967) Erythema multiforme in a premature infant associated with sepsis due to pseudomonas. *Pediatrics*, **39**, 120.

British Journal of Dermatology (1976) **95**, 187.

Studies on the effect of salicylic acid on normal skin

M.DAVIES AND R.MARKS

Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff

Accepted for publication 8 December 1975

SUMMARY

This investigation was prompted by our ignorance of the way in which salicylic acid aids desquamation. Salicylic acid in aqueous cream or white soft paraffin and in concentrations of 2–12% was applied to normal skin of twenty-three subjects while the vehicle alone was applied to the contralateral sites. Biopsies and skin surface biopsies were taken from the test and control sites after 1 week. Histological examination showed that there was less horny layer on the test specimens but that there were no qualitative or quantitative differences in the structure of the viable epidermis. Portions of the biopsies were incubated in the presence of tritiated precursor compounds and subsequent autoradiographic examination showed no difference in the incorporation of thymidine, cytidine or histidine between test and control preparations. Scanning electron microscopy of skin surface biopsies showed some changes in all specimens—presumably due to hydration—but also showed striking differences between test and control sites and were especially marked with higher concentrations of salicylic acid. It is suggested that salicylic acid causes desquamation by dissolution of intercellular cement material.

Salicylic acid is categorized as a 'keratolytic' and is used in a wide variety of skin disorders in which the only common factor seems to be the presence of an abnormal stratum corneum. Although it is an almost daily clinical observation that salicylic acid aids desquamation there is no evidence that it does so by lysing keratin. Keratin is a tough fibrous protein that remains in an intracellular position and it seems intrinsically unlikely that agents which promote desquamation do so by attacking this material. It is remarkable that a compound that is so widely prescribed and has such a broad therapeutic effect has promoted so little in the way of research into its mode of action. Strakosch (1943) claimed that it acted by producing epidermal irritation but there has been no substantiation of this finding and we can trace no other systematic investigation into its effects on the skin. In this study, we have examined the effects on normal skin of salicylic acid as usually prescribed, paying especial attention to any possible effect on the epidermis.

MATERIALS AND METHODS

Preparations used. The following preparations were used; 2, 4, 6, 8, 10 and 12% concentrations of salicylic acid in aqueous cream and 2, 6 and 10% salicylic acid in white soft paraffin.

Experimental design. The test material was applied to either the inner aspect of one arm or the lateral aspect of one thigh, and in order to make control observations, the contralateral site received the appropriate vehicle alone. Twenty-three subjects of mean age 40·2 years with a variety of common mild and benign disorders who had given their informed consent were used. Both test and control materials were applied twice daily, each application being rubbed into the test areas for 1 min on each occasion. The test areas were not occluded and no special instructions were given as to care of the sites between applications as it was intended to simulate the usual therapeutic regime.

After 1 week the treated areas were biopsied after anaesthetizing the sites with 1% lignocaine. The biopsies were divided into thin slices (1 mm thick) which were then incubated in Eagle's minimal essential medium at 37°C in an atmosphere of 5% CO_2 and 95% air for 2 h using a shaking incubator. The medium contained either tritiated thymidine,* tritiated cytidine or tritiated histidine in concentrations of 1 μCi/ml. After incubation the tissue slices were fixed in 10% formalin, dehydrated in alcohols and embedded in paraffin according to routine methods. Paraffin sections were dewaxed, dipped in nuclear emulsion (Ilford K2), exposed in dark boxes at 4°C for 2 week periods and developed in Kodak D19 developer. Haematoxylin and eosin stained autoradiographs from tissue incubated with tritiated thymidine were inspected for labelled basal and suprabasal epidermal cells. A cell was counted as 'labelled' if there were more than six grains over background over the nucleus. Labelling indices were calculated by counting between 500 and 1000 basal cells and expressing the labelled cells as a percentage of the total basal cells counted. The number of 'grains' per Malpighian cell in autoradiographs from tissue incubated with tritiated cytidine was estimated by assessing at least thirty Malpighian cells in 'evenly' labelled areas of randomly chosen portions of the section. The number of 'grains' per granular cell in autoradiographs from tissue incubated with tritiated histidine was estimated by assessing at least thirty granular cells in 'evenly' labelled areas of randomly chosen portions of the section.

Epidermal thickness was expressed in numbers of cells as the mean epidermal thickness (MET) after recording the numbers of cells comprising the thickness of the epidermis in a plane perpendicular to the basal lamina at twenty randomly chosen points. In some instances portions of pairs of test and control biopsies were snap frozen in liquid nitrogen and sectioned on a cryostat prior to treatment with a solution of 0·5% methylene blue according to Mackenzie & Linder (1973) in order to visualize the stratum corneum. To assess the effect of the preparations on the arrangement of the stratum corneum, skin surface biopsies (SSB's) were taken from the treated sites using a cyanoacrylate adhesive (Permabond, Staident Products) and the skin surface biopsy technique of Marks & Dawber (1971). These SSB's were cut to size and stuck to scanning electron microscope stubs before coating with a thin layer of carbon on an Edwards coating unit. The mounted specimens were then examined in a Cambridge scanning electron microscope (Mark IIb).

RESULTS

Sections from test and control biopsies were indistinguishable histologically. However, cryostat sections treated with the methylene blue solution demonstrated differences between test and control specimens. Specimens from salicylic acid treated sites had an irregular but overall much thinner stratum corneum than the control biopsies.

The MET's of test and control groups are given in Table 1. It can be seen that no significant differences were observed between any test and control group.

The L.I.s of autoradiographs derived from tissue incubated in the presence of tritiated thymidine are given in Table 2. It can be seen that there were no significant differences between any of the treatment and control groups. The labelling indices for the ointment and cream test groups were respectively 6·0±1·9 and 7·5±2·0 compared to values of 5·7±0·9 and 7·6±2·1 for their respective control groups. The ointment group controls had a labelling index of 5·7±0·9 compared to the cream group controls index of 7·6±2·1—a statistically significant difference ($P = 0{\cdot}001$).

There appeared to be no difference in the degree of labelling between test and control specimens incubated in the presence of tritiated histidine or cytidine (Figs 1 and 2). This was confirmed in the cases of 2% salicylic acid in cream and 10% salicylic acid in white soft paraffin by counting the number of grains per cell in the autoradiographs (Tables 3 and 4).

* Specific activities: thymidine = 18 Ci/mmole; cytidine = 29 Ci/mmole; histidine = 44 Ci/mmole.

TABLE 1. Mean epidermal thickness (in number of cells) after applying test and control preparations

Application		M.E.T. (±s.d.)	
		Test	Control
2	% salicylic acid in aqueous cream	5·3±0·3	4·8±0·3
4		5·1 (2)	5·2 (2)
6		6·4±2·4	5·0±0·7
10		6·8±2·2	4·9±0·1
12		5·1 (1)	4·8 (1)
2	% salicylic acid in white soft paraffin	5·3 (1)	4·2 (1)
6		4·8 (2)	4·8 (2)
10		5·0±0·6	5·0±0·6

TABLE 2. Labelling indices (percent basal and suprabasal cells labelled of total number of basal cells) after applying test and control preparations

Application		L.I. (±s.d.)	
		Test	Control
2	% salicylic acid in aqueous cream	7·0±1·5	8·2±0·5
4		6·4 (1)	7·2 (2)
6		7·7±2·2	8·5±3·3
10		7·6±2·2	6·4±2·0
12		9·6 (1)	8·0 (1)
2	% salicylic acid in white soft paraffin	8·0 (1)	5·8 (1)
6		5·5 (2)	5·7 (2)
10		5·7±2·1	5·8±0·8

Scanning electron microscopy of the skin surface biopsies demonstrated abnormalities in all specimens examined. These changes are demonstrated in Fig. 3 and include accentuation of scale margins, occasional slight gaps between scales, the appearance of slight thickening of some horny squames and the presence of a slight rippling pattern on some horn cells. In addition to these slight changes seen in all the specimens there were striking differences in the specimens that had received salicylic acid treatment. Firstly, there was marked irregularity in the overall arrangement of the horny layer (Fig. 4). Furrows due to skin surface markings were in general preserved but large gaps were evident in the specimens and there were many irregular and loose lamellae composed of several squames. Secondly, there was irregularity in scale apposition with large gaps (3–10 μm) between individual squames (Figs 5 and 6). In some specimens the gaps between horn cells obviously represented the sites of detached squames. The edges of the horn cells were sometimes irregular and even appeared serrated on occasions (Fig. 7). These changes were very much more obvious with the 6, 10 and 12% concentrations of salicylic acid.

COMMENT

It is surprising that, in an era of comparative pharmacological sophistication, so little is known of the pharmacology of the stratum corneum and virtually nothing about those compounds that promote desquamation. We have no doubt that salicylic acid 'works' and have attempted in this investigation to shed some light on how this is accomplished. Our results clearly indicate that the epidermis is not 'irritated' or driven to heightened epidermopoiesis by the application of salicylic acid as used in the

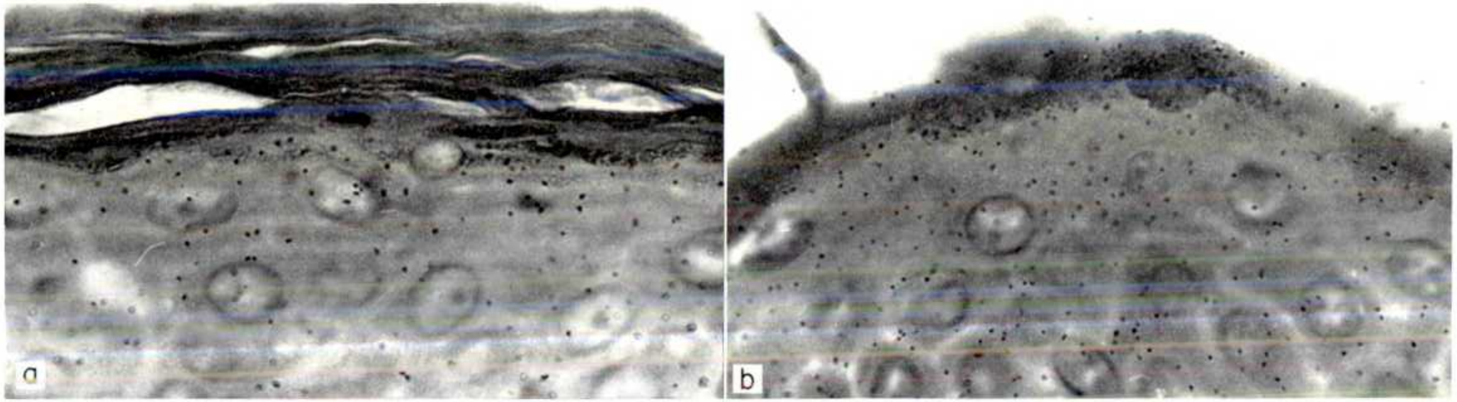


FIGURE 1. Autoradiographs from specimens previously incubated in the presence of tritiated histidine. (a) From control site. (b) From 6% salicylic acid in white soft paraffin treated site. There are more silver grains over the granular layers than in the Malpighian zones but there is no difference between control and test sites (H & E, × 400).

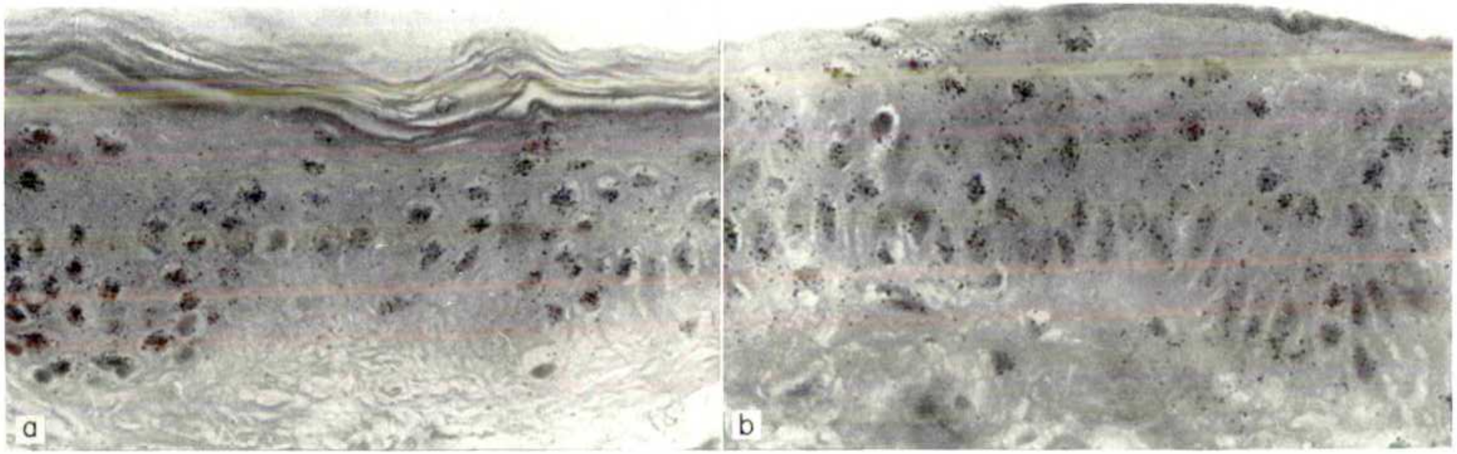


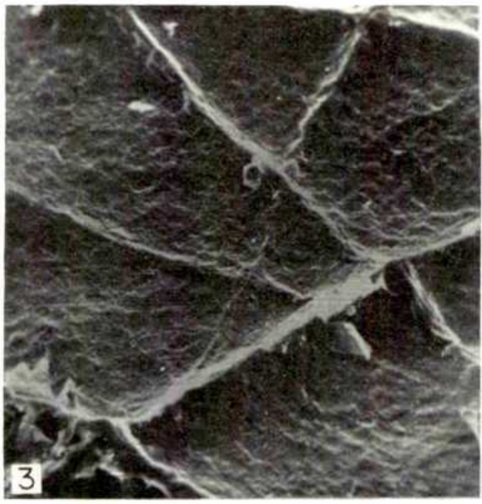
FIGURE 2. Autoradiographs from specimens previously incubated in the presence of tritiated cytidine. (a) From control site. (b) From 6% salicylic acid in white soft paraffin treated site. Silver grains are seen primarily over the nuclear areas but there is no difference in their numbers in the two specimens (H & E, × 400).

TABLE 3. Histidine incorporation (mean no. of grains/granular cell ± s.d.)

	Test	Control
2% salicylic acid in aqueous cream	9·2 ± 1·5	9·2 ± 2·8
10% salicylic acid in white soft paraffin	6·0 ± 2·2	5·3 ± 2·2

TABLE 4. Cytidine incorporation (mean no. of grains/Malpighian cell ± s.d.)

	Test	Control
2% salicylic acid in aqueous cream	13·8 ± 5·2	15·6 ± 6·5
10% salicylic acid in white soft paraffin	12·5 ± 1·8	16·7 ± 6·3



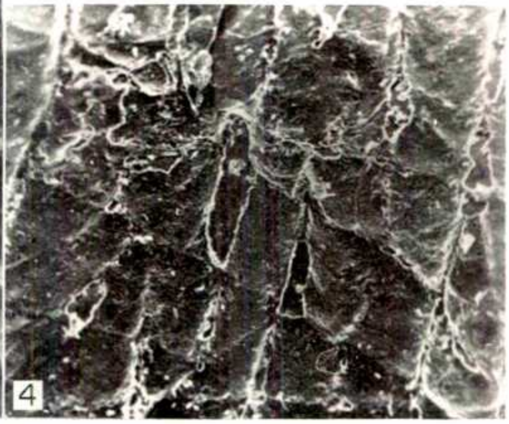
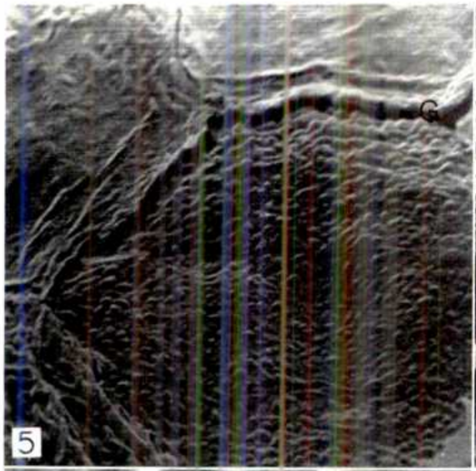


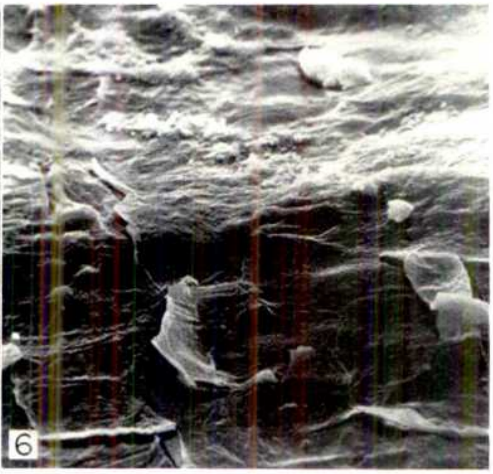
FIGURE 3. Scanning electron micrograph of a skin surface biopsy taken from control site to show accentuation of scale margins (× 200).

FIGURE 4. Scanning electron micrograph of skin treated with 6% salicylic acid in white soft paraffin showed marked irregularity in the overall arrangement of the horny layer (compare with Fig. 3) (× 100).

treatment of many dermatoses. Nonetheless, it is possible that salicylic acid may irritate the epidermis when applied for longer periods of time or when used more frequently under occlusion. The cryostat sectioned specimens indicate that the horny layer has detached (or was more readily detachable) from the skin treated with salicylic acid suggesting that there was either enhanced desquamation *in vivo* or easier detachment during section preparation.

Scanning electron microscopy of all specimens showed some separation between individual squames although this was much more marked in the 'test samples'. Probably this effect is partially due to hydration from the 'occlusive' nature of the topical preparations used. It is a well known fact that wet stratum corneum swells and is more easily detached from the skin. However, the major part of the spacing between squames produced by the salicylic acid preparations could only have been due to salicylic acid. In some specimens, especially those treated by the higher concentrations, this separation resulted in large sections of the stratum corneum becoming detached and the appearance of disorganization of the specimens.





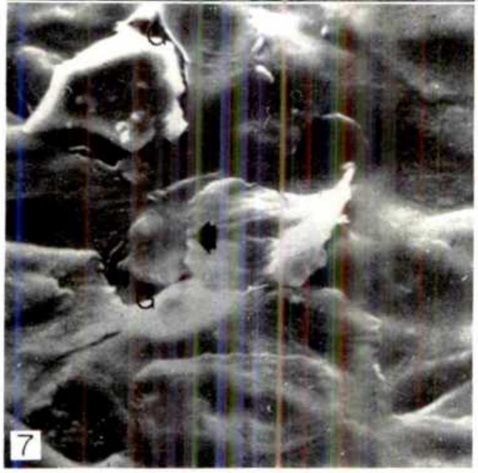


FIGURE 5. Scanning electron micrograph of skin surface biopsy taken from a site treated with 10% salicylic acid in white soft paraffin showing a gap—approximately 3 μm in width(G) (× 2200).

FIGURE 6. This scanning electron micrograph is of a skin surface biopsy taken from a site treated with 6% salicylic acid in white soft paraffin and shows loose and detaching squames and gaps (× 1100).

FIGURE 7. Large gaps (G) are evident in this micrograph of a skin surface biopsy taken from a 6% salicylic acid in white soft paraffin treated site. Some of the scale margins are unusually serrated (↑) (× 1400).

It seems not unreasonable to suggest from our findings that salicylic acid preparations enhance desquamation by encouraging squame separation by causing the dissolution of intercellular cement material. Work is now in progress to test this hypothesis.

REFERENCES

MARKS, R. & DAWBER, R.P.R. (1971) Skin surface biopsy. An improved technique for examination of the stratum corneum. *British Journal of Dermatology*, **84**, 117.

MACKENZIE, I.C. & LINDER, J.E. (1973) An examination of cellular organization within the stratum corneum by a silver staining method. *Journal of Investigative Dermatology*, **61**, 245.

STRAKOSCH, E.A. (1943) Studies on ointments. II. Ointments containing salicylic acid. *Archives of Dermatology and Syphilology*, **47**, 16.

British Journal of Dermatology (1976) **95**, 193.

Dithranol (anthralin) versus triacetoxyanthracene

INVESTIGATIONS *IN VITRO*

WOLFGANG P.RAAB

Department of Medical Chemistry, Vienna University Medical School,
Waehringerstrasse 10, A-1090 Vienna, Austria

Accepted for publication 18 November 1975

SUMMARY

In two *in vitro* models for evaluating antipsoriatic activity, dithranol (anthralin) and triacetoxyanthracene were compared. Both compounds exhibited an about equal effect on oxygen consumption of surviving human skin. Dithranol, however, exerted a stronger inhibiting effect on glucose-6-phosphate dehydrogenase (G-6-PDH) activity of human skin homogenates.

Effective antipsoriatic agents have been shown to decrease oxygen consumption of surviving human skin and to inhibit the pentose phosphate shunt in human skin homogenates (Raab, 1969, 1975; Raab & Gmeiner, 1975). Both types of action are important and serve to calculate the 'anti-psoriatic index' (Raab, 1975). Although decreased oxygen consumption and inhibition of the pentose phosphate shunt are connected, the inhibition of glucose-6-phosphate dehydrogenase (G-6-PDH) activity deserves special attention in regard to anti-psoriatic activity.

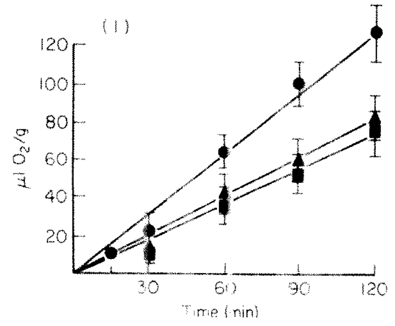
Dithranol (anthralin) was found to decrease oxygen consumption of surviving human skin (Raab, 1969) and to inhibit G-6-PDH activity in human skin homogenates (Raab & Siber, 1974). To avoid the staining and irritant action of dithranol in clinical therapy (Raab, 1975; Hellier & Whitefield, 1967), a triacetoxy derivative has been developed (Kammerau, Zesch & Schaefer, 1975) which, however, was found to be less effective in psoriasis. The following paper deals with a comparison between dithranol and triacetoxyanthracene in two biochemical assays which may be used as *in vitro* models for anti-psoriatic activity.

MATERIALS AND METHODS

Oxygen consumption. Human skin samples from fresh corpses of young adults were cleaned from adjacent tissues, washed with iced 0·15 mol/l NaCl and cut into portions weighing 200 to 300 mg. These pieces were dissected into thin slices and introduced into the main compartment of sterile Warburg flasks. The respiratory medium in the main compartment of these flasks consisted of 2·7 ml of Ringer solution (pH 7·4), 0·2 ml of 5% glucose in twice distilled water, and 0·1 ml of dimethylformamide. In the controls, plain dimethyl formamide was used; in the test assays, dimethylformamide containing either dithranol or triacetoxyanthracene was introduced. The central compartment of the Warburg flasks contained 0·2 ml of a 20% KOH solution to absorb carbon dioxide. The gas phase was air. The flasks were fixed to the manometers and hung in a water-bath at a temperature of 37°C. After an adaption period of 10 min, flasks and manometers were closed and recording of oxygen consumption was started (readings every 15 min over 2 h). Altogether, six different experiments with twenty-four flasks each were evaluated.

G-6-PDH-activity. Fresh human skin samples were separated from adherent tissue, washed repeatedly with 0·15 mol/l NaCl, and cut into small pieces. These pieces were homogenized with iced 0·15 mol/l NaCl with an Ultraturrax blender and centrifuged in the cold (4500 rev/min for 5 min). To 2·9 ml of the supernatant (protein content 0·55±0·08 g% as determined by the Biuret method) 0·1 ml of either plain dimethylformamide or dimethylformamide containing either dithranol or triacetoxyanthracene was added. In these series, final concentrations of the drugs amounted to 0·05, 0·5, and 1·0 mmol/l. After stirring, the test tubes were incubated at 37°C for 2 h. Thereafter, 1·0 ml was withdrawn from tests and controls and was added to 2·0 ml of a 50 mmol/l triethanolamine buffer (pH 7·6) containing 5 mmol/l ethylenediaminetetraacetate, 0·05 ml of a 31 mmol/l glucose-6-phosphate and 0·1 ml of a 10 mmol/l NADP. At 25°C, extinction was read immediately after stirring and 1, 2 and 3 min later. The mean difference in extinction per min was used to calculate mU G-6-PDH-activity (EC 1.1.1.49) per ml in the controls and in the tests (multiplication with 506). Seven different preparations were evaluated in this part of the study.

Test substances. Dithranol (M = 226) and triacetoxyanthracene (M = 352) were dissolved in dimethylformamide. 0·1 ml of these solutions were added to 2·9 ml of Ringer/glucose containing skin slices in the Warburg flasks or to 2·9 ml of human skin homogenate.



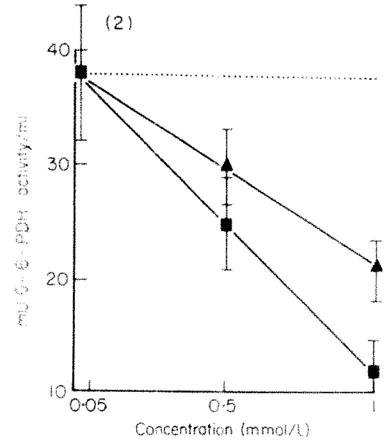


FIGURE 1. Oxygen consumption of surviving human skin in the presence of dithranol (10 μg/ml = 46 μmol/l) (■——■); triacetoxyanthracene (15 μg/ml = 43 μmol/l) (▲——▲); control ●——●.

FIGURE 2. G-6-PDH-activity of human skin homogenates following incubation with dithranol (■——■) and triacetoxyanthracene (▲——▲) in various concentrations. Control · · · · · ·.

RESULTS

Oxygen consumption. In the controls, oxygen consumption measured 62±8 μl O$_2$ per g skin per h. Over the 2-h period of the experiments, oxygen consumption appeared linear (Fig. 1). Dithranol in a concentration of 46 μmol/l (= 10 μg/ml) reduced the oxygen consumption to 79±13 μl O$_2$ per g fresh weight over the 2-h period. Similarly, triacetoxyanthracene in a concentration of 50 μmol/l (= 15 μg/ml) reduced the oxygen consumption to 82±15 μl O_2 per g fresh weight over the 2-h period. In both instances, the reduction in oxygen consumption was statistically significant ($P<0{\cdot}01$). Lower concentrations of dithranol or triacetoxyanthracene (5 μmol/l) remained without significant influence on oxygen consumption by surviving human skin.

G-6-PDH activity. G-6-PDH-activity in the human skin homogenates (protein content 0·55 g% as determined by the Biuret method) measured 38±6 mmol/ml. After incubation with dithranol or triacetoxyanthracene in concentrations above 0·5 mmol, G-6-PDH-activity was significantly decreased ($P<0{\cdot}01$). Details are shown in Fig. 2.

COMMENT

It has been pointed out in a recent survey (Raab, 1975) that dithranol exerts its anti-psoriatic effects in three ways which are, in some respects, closely related:

Enzymic inhibition (direct inhibition or reaction with important coenzymes).

Reaction with nucleic acids and disturbance of enzymes important in nucleic acid metabolism (see Liden & Michaëlsson, 1975; Fisher & Maibach, 1975).

Inhibition of mitosis and interference with general cell metabolism (inhibition of cell respiration, cytostatic effects, cytotoxic actions, elicitation of mutations).

References to the various actions of dithranol, particularly these three 'antipsoriatic' effects, may be found in the above-mentioned survey (Raab, 1975).

In psoriatic lesions, enzymes of the pentose–phosphate shunt show an activity 4 times normal; tissue respiration, as judged by oxygen consumption, reaches twice the normal values (Raab, 1975; Raab & Gmeiner, 1975). These facts underline the importance of special biochemical effects (inhibition of G-6-PDH-activity) of anti-psoriatic drugs, in addition to a rather non-specific inhibition of tissue respiration. It has been claimed repeatedly (Raab, 1975) and confirmed again in a recently published series of investigations (Cotton & van Rossum, 1975) that the inhibition of G-6-PDH-activity is a good indicator for screening anti-psoriatic efficacy.

Topical (dithranol, triacetoxyanthracene, 6-hydroxy-2-oxobenzoxathiol) as well as systemic (azathioprine, methotrexate) 'antipsoriatic' drugs have been shown to inhibit tissue respiration of surviving human skin and to block the pentose–phosphate shunt. However, the drugs listed above differ markedly in their relative strengths as judged in the two models described here.

Dithranol and triacetoxyanthracene both decrease oxygen consumption of surviving human skin and inhibit G-6-PDH activity in human skin homogenates. On a molar basis, the influences of the two compounds on oxygen consumption are almost identical. In its enzyme-inhibiting effect, however, triacetoxyanthracene was much less active than dithranol.

Clinically, triacetoxyanthracene was found to be less effective in psoriasis than is dithranol. The fact that the maximal penetration rate of triacetoxyanthracene is less (0·04 μg/cm^2) than that of dithranol (0·3 μg/cm^2) is partially countered by the fact that triacetoxyanthracene accumulates more in the deeper skin layers. Maximal dermal concentrations of triacetoxyanthracene are about double (0·1 μg/cm^3) the maximal concentrations of dithranol (0·045 μg/cm^3) (Kammerau *et al.*, 1975). Here, too, the differences in molecular weight must be taken into consideration. The results of the present study permit the assumption that the weaker anti-psoriatic action of triacetoxyanthracene is due to a lower biochemical–pharmacological activity.

REFERENCES

COTTON D.W.K. & VAN ROSSUM, E. (1975) Psoriatic therapeutics and glucose-6-phosphate dehydrogenase. *Archiv für Dermatologische Forschung* **252**, 147.

FISHER, L.B. & MAIBACH, H.I. (1975) The effect of anthralin and its derivatives on epidermal cell kinetics. *Journal of Investigative Dermatology*, **64**, 338.

HELLIER, F.F. & WHITEFIELD, M. (1967) The treatment of psoriasis with triacetoxyanthracene. *British Journal of Dermatology*, **79**, 491.

KAMMERAU, B., ZESCH, A. & SCHAEFER, H. (1975) Absolute concentrations of dithranol and triacetyl-dithranol in the skin layers after local treatment: *in vivo* investigations with four different types of pharmaceutical vehicles. *Journal of Investigative Dermatology*, **64**, 145.

LIDEN, S. & MICHÄELSSON, G. (1974) Dithranol (anthralin) in psoriasis. The effect on DNA-synthesis, granular layer, and parakeratosis. *British Journal of Dermatology*, **91**, 447.

RAAB, W. (1969) Die Wirkung externer Antipsoriatica auf die Gewebsatmung menschlicher und tierischer Haut. *Archiv für klinische und experimentelle Dermatologie*, **234**, 44.

RAAB, W. (1975) Zur antipsoriatischen Wirkung von Dithranol. 1. und 2. Teil. *Hautarzt*, **26**, 452 & 456.

RAAB, W. & GMEINER, B. (1975) *In vitro* evaluation of methotrexate and azathioprine for antipsoriatic activity. *Archives of Dermatology Research*, **253**, 77.

RAAB, W. & SIBER H. (1974) Glucose-6-phosphat-Dehydrogenase und externe Antipsoriatica. *Archiv für Dermatologische Forschung*, **249**, 179.

British Journal of Dermatology (1976) **95,** 197.

Case Reports

Mudi-chood on the pinnae

P. SUGATHAN

Department of Dermatology and Venereology, Medical College Hospital, Calicut—673008, India

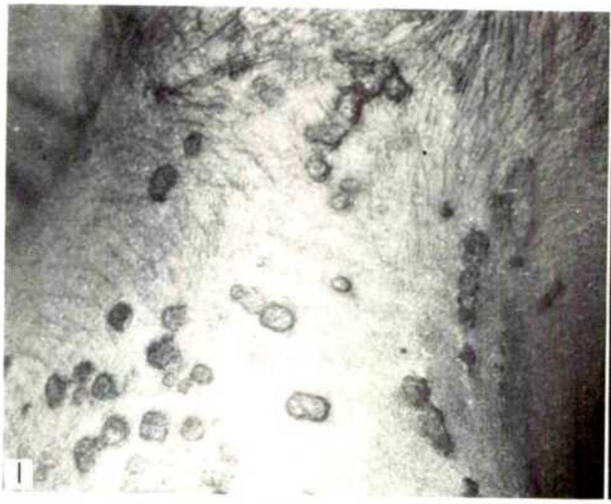
Accepted for publication 1 December 1975

SUMMARY

A case of Mudi-chood on the pinnae in a young girl is reported.

'Mudi-chood'* is a peculiar dermatosis that affects girls and young women in Kerala State, South India. It is characterized by sharply defined, coin-shaped, flat-topped, brownish-black papules on the nape of the neck and upper exposed parts of the back (Fig. 1). In a series of sixteen cases originally reported by Sugathan & Nair (1972) no other site was found to be affected, but we recently observed typical lesions of Mudi-chood on the pinnae of a young girl.

The patient, Miss L.J., was a 17-year-old college student who attended the out-patient section of the Department of Dermatology and Venereology, Medical College Hospital, Kottayam. She com-



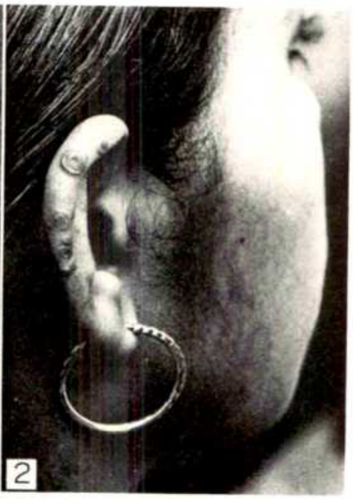


FIGURE 1. Typical lesions of Mudi-chood on the nape of the neck. Note Köbner effect.

FIGURE 2. Lesions of Mudi-chood over the right pinna.

---

* Mudi = hair, Chood = heat, from the local belief that the heat of the hair is responsible.

plained of mildly pruritic, pigmented papules on both pinnae. The lesions had started 6 months previously and were at first asymptomatic. She sought medical advice only when they began to itch.

On examination she was a healthy, fair girl of medium build. On the postero-lateral aspects of the upper halves of both pinnae there were a few discrete, sharply defined moderately pigmented papules (Fig. 2). No scratch marks or Köbner effect were evident. The papules had a thin keratinous rim. She had long, black rather coarse hair. Her hair was habitually dressed to cover the entire external ears except the lobes. There was no evidence of any other disease. She refused a biopsy examination. Clinical response to non-specific topical medication was satisfactory.

Typical lesions of Mudi-chood are seen only on the nape of the neck and upper exposed parts of the back. Alteration of the microclimate of the affected skin due to contact with moist, oily hair in a humid environment was postulated as having a possible aetiological role in this disease. It now appears that it may also occur over the pinnae in women who habitually cover the external ears with their hair.

ACKNOWLEDGMENTS

Thanks are due to Dr J.Marshall, Professor of Dermatology, Stellenbosch University, South Africa, for correcting the manuscript and the Superintendent, Medical College Hospital, Kottayam, for permission to use hospital records.

REFERENCE

SUGATHAN, P. & NAIR, M.B. (1972) Mudi-chood—a new dermatosis. In: *Essays on Tropical Dermatology* (Ed. by J.Marshall), Vol. 2, p. 183. Excerpta Medica, Amsterdam.

British Journal of Dermatology (1976) **95**, 199.

Multiple pigmented naevi in an albino

M.HALL, V.EUSEBI* AND I.D'ANGELO†

Department of Histopathology, Hammersmith Hospital, Du Cane Road, London W12, and † Ospedale Regionale 'Umberto 1', Reparto Dermatologia, Ancona, Italy

Accepted for publication 29 December 1975

SUMMARY

A case of pigmented naevi arising in an albino is described. The histological appearance of a naevus is described and the nature of the pigment discussed.

Ephelides, naevi and malignant melanomas have been reported in albinos (Ito, Noguchi & Komatsu, 1956; Barnicot, 1952; Young, 1957; Kennedy & Zelickson, 1963; Duron, 1965; Garrington *et al.*, 1967). With the exception of ephelides occurring in African albinos, in none of these reported cases have the lesions been pigmented. The case reported here is of an albino with multiple pigmented naevi. Pigment was present in one lesion biopsied and was shown to be melanin by conventional staining methods.

CASE REPORT

The patient was a 7-year-old Italian female in whom albinism had been diagnosed at birth. Both parents showed normal pigmentation and there was no known family history of albinism. The child had never tanned on exposure to sunlight but had suffered first and second degree burns. At the age of 9 months she developed strabismus. When 5-years-old she developed a few pinkish-yellow areas on her back, which increased in size and some subsequently developed central dark brown pigmentation. Over the next 2 years more of these areas appeared, some developing pigmentation. At the time of presentation many such lesions were present on her trunk and limbs (Fig. 1). The child was of normal intelligence and general physical examination was normal. Ophthalmological examinations revealed decreased visual acuity, a slow horizontal nystagmus, absent pigment in the fundus with a red pupil and a slight grey colour of the iris.

Routine haematological and biochemical tests were within normal limits and no peroxidase-positive inclusions such as are seen in Chediak-Higashi disease were seen in peripheral blood leukocytes. Chromosome studies on peripheral blood leukocytes showed a normal female pattern. Tyrosinase activity was not measured. A clinical diagnosis of oculo-cutaneous albinism was made.

One of the brown lesions was excised. Histologically it showed features of both a compound and a blue naevus (Fig. 2). The intradermal component of the compound naevus blended into an area

---

* Present address: 1st Anat Patologica, Ospedale S. Orsola, Bologna, Italy.

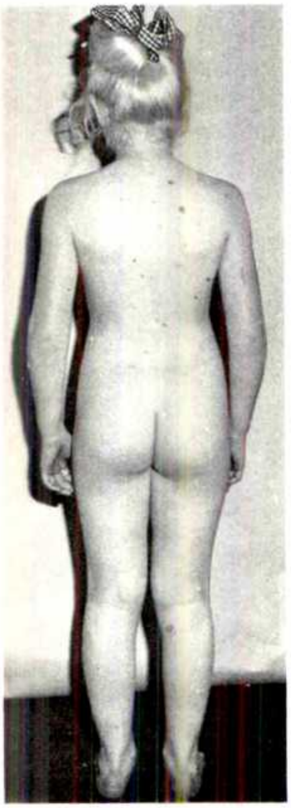
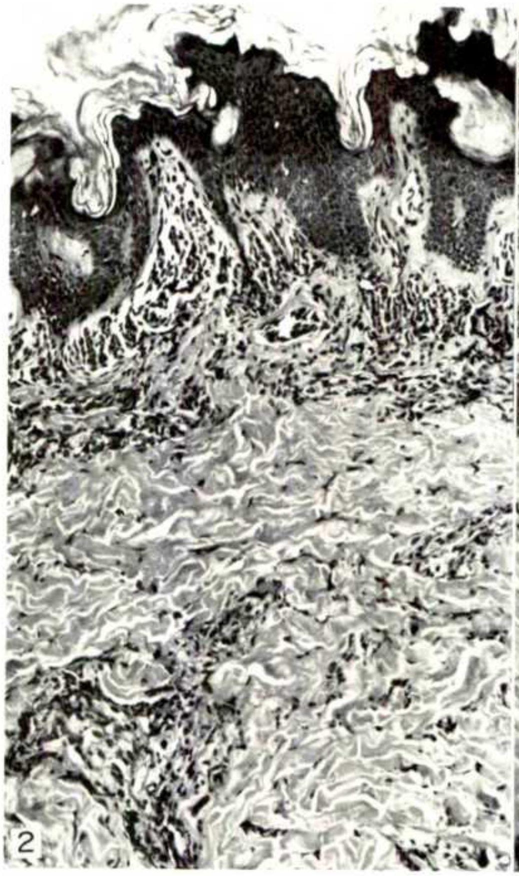


FIGURE 1. Patient showing pigmented lesions on trunk, legs and arms.

composed of spindle cells, mainly grouped around adnexa and dermal vessels, which was the blue naevus component of the lesion. Brown pigment was present in the naevus cells and surrounding macrophages. The pigment was abundant in the cells of the blue naevus and intradermal components of the lesion, and was present as occasional fine granules in the junctional component. The pigment was bleached on exposing the section to acidified 0·5% potassium permanganate for 7 min and was positively stained by the Masson Fontana and Schmorl techniques for melanin (Fig. 3). The Perl technique did not stain the pigment. These methods identify the pigment as melanin (Pearse, 1972). No pigment was demonstrated in the epidermis above or away from the lesion by any of the staining methods used.

DISCUSSION

Previous reports of benign naevi (Ito *et al.*, 1956) and malignant melanomas (Duron, 1965; Young, 1957; Kennedy & Zelickson, 1963; Garrington *et al.*, 1967) occurring in albinos have failed to demonstrate melanin pigmentation. To our knowledge no blue naevi have been reported. The present case appears unique in that abundant melanin was present mainly in the blue naevus cells and in surrounding macrophages. Though tyrosinase activity was not measured, the clinical picture of non-pigmented skin, hair and eyes with marked visual disturbance and undue erythema on exposure to sunlight, strongly support the clinical diagnosis of oculo-cutaneous albinism.



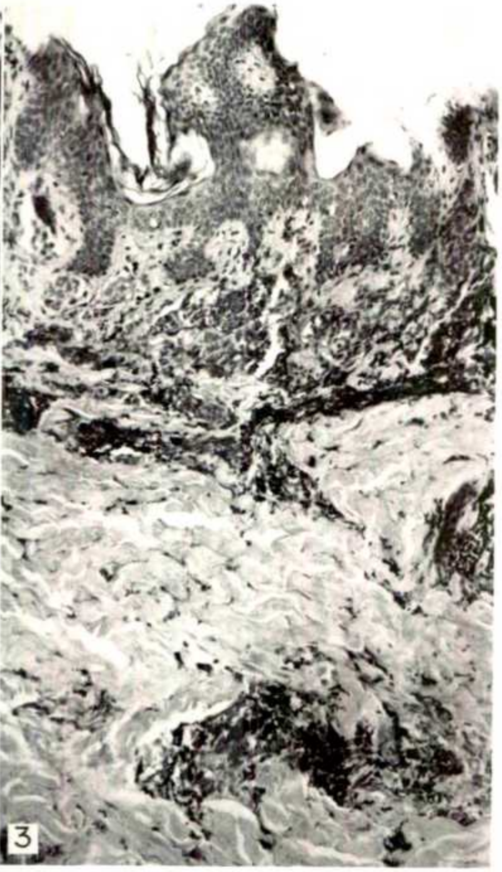


FIGURE 2. Photomicrograph of pigmented lesion showing superficial and deep components of the lesion (H & E, × 150).

FIGURE 3. Photomicrograph of pigmented lesion showing melanin pigmentation (Masson Fontana, × 150).

It is known that melanin pigmentation in the form of ephelides can occur in albinos (Barnicot, 1952) and in the present case melanin has been demonstrated in a naevus occurring in an albino. It would appear that in a proportion of cases of oculo-cutaneous albinism, though normal melanocytes do not produce melanin, the abnormal melanocytes in ephelides and naevi can overcome the genetic defect in melanogenesis and produce melanin pigment.

ACKNOWLEDGMENTS

We are grateful to Professor J.G.Azzopardi for helpful discussion of this case, to Miss Y.Maddison for technical assistance and to Mr W.Hinks for photographic help.

REFERENCES

BARNICOT, N.A. (1952) Albinism in south-western Nigera. *Annals of Eugenetics*, **17**, 38.

DURON, R.A. (1965) Melanoma maligno del albino. *Revista medica Hondurena*, **33**, 149.

GARRINGTON, G.E., SCOFIELD, H.H., CORNYN, J. & LACY, G.R., JR. (1967) Intra-oral malignant melanoma in a human albino. *Oral surgery, oral medicine and oral pathology*, **24**, 224.

ITO, M., NOGUCHI, J. & KOMATSU, A. (1956) 'Lentigo achromiant' chez les albinos. *Annales de Dermatologie*, **83**, 631.

KENNEDY, B.J. & ZELICKSON, A.S. (1963) Melanoma in an albino. *Journal of the American Medical Association*, **186**, 839.

PEARSE, A.G.E. (1972) *Histochemistry Theoretical and Applied*, 3rd edn, Vol. **2**, p. 1050. Churchill Livingstone, London.

YOUNG, T.E. (1957) Malignant melanoma in an albino. *Archives of Pathology*, **64**, 186.

British Journal of Dermatology (1976) **95**, 203.

Leg ulcers in a family with both beta thalassaemia and glucose-6-phosphate dehydrogenase deficiency

S.GANOR AND T.COHEN

Outpatient Department of Dermatology and Venereology, Kupath Holim, and Department of Human Genetics, Hadassah University Hospital, Jerusalem, Israel

Accepted for publication 7 November 1975

SUMMARY

A family is presented in which the proposita, affected with thalassaemia major, developed a chronic leg ulcer at the age of 14 years. Her eldest brother, not affected with thalassaemia, had a transient leg ulcer at the age of 18 years and a second brother, affected with thalassaemia minor, developed leg ulcers when aged 15 years. All three siblings were glucose-6-phosphate dehydrogenase deficient.

Leg ulcers, a rare observation in children and young adults, may be precipitated by environmental and hereditary factors. A common environmental cause is trauma to the skin (Rook, Wilkinson & Ebling, 1972), while a well-known hereditary cause is sickle-cell anaemia (Lehmann & Huntsman, 1966). Thalassaemic leg ulcers have been described by Estes, Farber & Stickney (1948), Marmont & Bianchi (1948), March, Schlyen & Schwartz (1952), Pascher & Keen (1952), Cooper & Wacker (1954), Zelickson (1957), Pascher & Keen (1957), Samitz, Waldorf & Shrager (1964), Montagnani, Pisani & Argenziano (1966), Berge, Brehmer-Andersson & Rorsman (1970). We wish to describe a family in which several members are affected with a combination of thalassaemia minor or major, glucose-6-phosphate dehydrogenase (G6PD) deficiency and leg ulcers.

CASE REPORTS

Family history

The proposita, of Jewish Kurdish descent, was hospitalized repeatedly during a 7-year period because of a persistent ulcer on the inner aspect of the right ankle. Of thirteen children in the family, five died in infancy or early childhood in Iraq of unknown causes and eight are alive (Fig. 1). The father is affected with classical beta thalassaemia minor while the mother showed raised levels of haemoglobin A_2 on repeated examinations with no additional haematological manifestations suggestive of beta thalassaemia.

The proposita, born in 1952 in Israel, was severely anaemic at 15 months of age. Upon hospitaliza-

Reprint requests: Dr S.Ganor, 23 Keren Hayesod str., Jerusalem 94188, Israel.

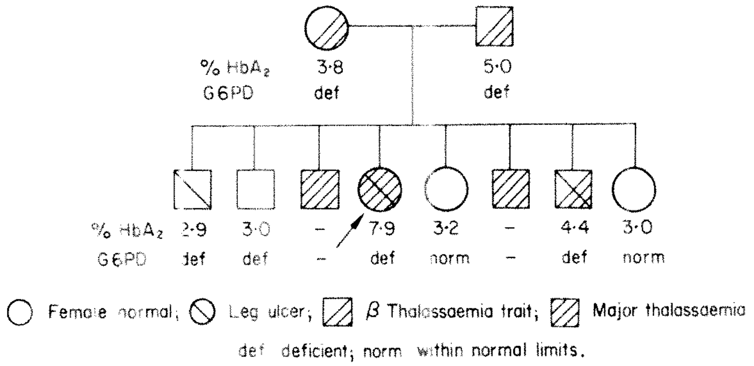

% HbA2
G6PD
3·8
def
5·0
def
% HbA2
2·9
def
3·0
def
–
–
7·9
def
3·2
norm
–
–
4·4
def
3·0
norm
Female normal; Leg ulcer; β Thalassaemia trait; Major thalassaemia
def deficient; norm within normal limits.

FIGURE 1. Pedigree of the living members of the family.

tion, thalassaemia major was diagnosed. In that and subsequent years she received repeated transfusions when her haemoglobin level reached 4·5–6·8 g%.

In 1966 she was hospitalized for a sharp-bordered elongated ulcer on the right inner ankle, approximately 3 months after a puncture wound at that site. In 1968 when the ulcer became recalcitrant to treatment, she was referred to the Dermatology Department. She was then in generally good health. Skin and sclerae were pale, slightly icteric. Heart and lungs were normal; the liver was not palpable and the spleen barely palpated below the costal margin. No varicose veins in the lower limbs were present. The dorsalis pedis and tibialis posterior arteries were palpable. Blood pressure was 140/90 mmHg. At and above the right inner ankle a 4 × 2·5 cm, oval, shallow ulcer was present. Its borders were sharp, somewhat elevated; its floor, about 2 mm deep, was covered with granulation tissue.

Laboratory examinations. Urine and stool normal. Haemoglobin 6·4 g%, haematocrit 24%; hypochromia, anisocytosis, microcytosis, poikilocytosis and target cells were found. Osmotic fragility of RBC was decreased; Hb A_2 7·9%, fetal haemoglobin 11·8% and glucose-6-phosphate dehydrogenase (G6PD) deficiency of RBC was demonstrated. Protein electrophoresis: albumin 47·3%, alpha$_1$ globulin 3·4%, alpha$_2$ globulin 8·2%, beta globulin 7·8% and gamma globulin 33·2%; bilirubin 1·9 mg% and prothrombin time 34%. Capillaroscopy showed very pale small blood vessels with aggregation of red blood cells and narrowed capillaries in the conjunctivae. Oscillography and oscillometry of the lower limbs were normal. Histological examination of the ulcer was non-specific.

In 1969 the ulcer closed completely with local applications of O_2 three times a day and 0.25% aqueous $AgNO_3$. In 1971, during an episode of fever and chills, the ulcer re-opened (Fig. 2) and an abscessed spleen was removed. Upon recovery the ulcer remained about 0·5 cm in diameter until her first pregnancy, when the ulcer enlarged and deepened.

The eldest brother of the proposita, born in 1940 (Fig. 1), was hospitalized in the Dermatology Department because of a leg ulcer at the age of 28 years. He had had gastric complaints since 1958. Ten months before his admission itching and scaling were noticed at the inner aspect of the left ankle. Within 1 month this had developed into a shallow purulent indolent ulcer (20 × 15 mm) surrounded by erythema. Its sharp borders were neither infiltrated nor undermined. Varices were not present and the dorsalis pedis artery was palpated.

Laboratory examinations. Urine, stool, RBC, WBC and routine blood chemistry normal, haemoglobin A_2 2·9%, haemoglobin F 0·5%. G6PD deficiency was present.

A younger brother of the proposita (Fig. 1), born in 1957 in Israel, is affected with beta thalassaemia

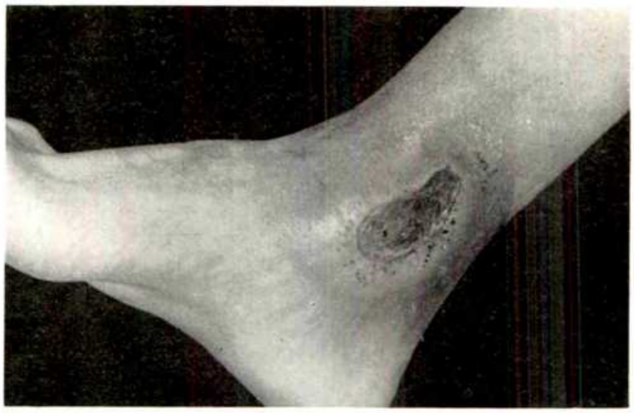


FIGURE 2. Leg ulcer of proposita in 1971.

minor: haemoglobin 9·4g%, haematocrit 30%, haemoglobin A_2 4·4%, haemoglobin F 12·6%, morphology of RBC typical for thalassaemia. He has G6PD deficiency. In October, 1972, he presented at the out-patient Dermatology Clinic with a 12 × 12 mm indolent purulent ulcer with sharp, not elevated or undermined borders on the left medial malleolus. It closed within 1 month with local treatment. In September 1973, a similar ulcer appeared after trauma to the same site and healed within 3 months.

COMMENT

Thirteen patients with beta thalassaemia minor and leg ulcers have been reported (Berge *et al.*, 1970), without any data on G6PD activity of the patients. G6PD deficiency is common (60%) among Jews from Kurdistan (Cohen, 1971).

The report of this family raises the question of the connection between thalassaemia, G6PD deficiency and leg ulcers in young patients. In this family both parents are G6PD deficient, but the mother is heterozygous since she has two phenotypically normal daughters. All the three siblings with leg ulcers are G6PD deficient. The healing of the leg ulcers was good in the brother without thalassaemia and no recurrences occurred. However, recurrences occurred in the brother affected with thalassaemia minor, and in the proposita with thalassaemia major the ulcer is recalcitrant. The possibility is considered that G6PD deficiency is a contributing factor in the pathogenesis of leg ulcers in young people, while the hypoxia due to the thalassaemia enhances this trend.

ACKNOWLEDGMENTS

We thank Professor E.Davis for capillaroscopic examination and Professor F.Sagher for histological examination.

REFERENCES

BERGE, G., BREHMER-ANDERSSON, E. & RORSMAN, H. (1970) Thalassemia minor and painful ulcers of lower extremities. *Acta dermato-venereologica*, **50**, 125.

COHEN, T. (1971) Genetic markers in migrants to Israel. *Israel Journal of Medical Sciences*, **7**, 1509.

Cooper, C.D. & Wacker, E.C. (1954) The successful therapy with streptokinase-streptodornase of ankle ulcers associated with mediterranean anaemia. *Blood*, **9**, 241.

Estes, J.E., Farber, E.M. & Stickney, J.M. (1948) Ulcers of the leg in mediterranean disease. *Blood*, **3**, 302.

Lehmann, H. & Huntsman, R.G. (1966) *Man's Haemoglobins*. North-Holland Publishing Co., Amsterdam.

March, H.W., Schlyen, S.M. & Schwartz, S.E. (1952) Mediterranean hemopathic syndrome (Cooley's anemia) in adults. *The American Journal of Medicine*, **13**, 46.

Marmont, A. & Bianchi, V. (1948) Mediterranean anaemia. *Acta Haematologica*, **1**, 4.

Montagnani, A., Pisani, M. & Argenziano, G. (1966) Hautschadigungen im Verlaufe von Hämoglobinopathien. *Der Hautarzt*, **17**, 201.

Pascher, F. & Keen, R. (1952) Chronic ulcers of the leg associated with blood dyscrasias. *Archives of Dermatology and Syphilology*, **66**, 478.

Pascher, F. & Keen, R. (1957) Ulcers of the leg in Cooley's anemia. *New England Journal of Medicine*, **256**, 1220.

Rook, A., Wilkinson, D.S. & Ebling, F.J.G. (1972) *Textbook of Dermatology*, 2nd edn, p. 479. Blackwell Scientific Publications, Oxford.

Samitz, M.H., Waldorf, D.S. & Shrager, T. (1964) Leg ulcers in mediterranean anaemia. *Archives of Dermatology*, **90**, 567.

Zelickson, A.S. (1957) Leg ulcers associated with mediterranean anaemia (thalassemia major and minor). *Archives of Dermatology*, **76**, 351.

British Journal of Dermatology (1976) **95**, 207.

Glaucoma following the application of corticosteroid to the skin of the eyelids

R.BEVIS CUBEY

Sunderland Eye Infirmary

Accepted for publication 21 November 1975

SUMMARY

A case is reported of intractable glaucoma presumed to be associated with the long-term use of a corticosteroid-containing dermatological preparation on the skin of the eyelids for the control of eczema.

CASE HISTORY

A 22-year-old man presented at an ophthalmic clinic with a 6-week history of seeing coloured haloes when he looked at a bright light, with constant watering of both eyes and slight hazing of his vision. The cause of these symptoms was found to be corneal oedema due to severely elevated intra-ocular pressure (60 mmHg) in both eyes. Normal intra-ocular pressure was restored only after 5 days of intensive medical therapy in hospital. As he did not exhibit either cupping or atrophy of the optic discs, nor any glaucomatous defects in his visual fields, nor any evidence of closure of the irido-corneal angle, glaucoma surgery was not performed at this stage. However, even after 11 months it proved impossible to control his intra-ocular pressures, with the resulting visual symptoms and ocular irritation, by medical measures on an out-patient basis. Therefore, despite the continued absence of any evidence of permanent ocular damage, bilateral glaucoma filtration operations were performed with subsequent control of both his intra-ocular pressures and his symptoms, with 6/6 vision in each eye.

No cause was found for his glaucoma, and he showed none of the features of congenital glaucoma of late onset nor of chronic simple glaucoma of early onset. Initial direct questioning elicited no history of medication either systemically or into the eyes, but subsequent specific enquiry revealed that he had applied fluocinolone acetonide ointment 0·01% to the skin of his face, including the eyelids, every night for some 7 years to control his facial eczema, a diagnosis (though not a line of therapy) suggested by a dermatologist some 10 years earlier, and now further complicated by typical corticosteroid-induced epidermal atrophy with telangiectatic erythema of the entire face (Sneddon, 1974).

DISCUSSION

Corticosteroid-induced glaucoma is a well-documented entity not uncommonly encountered by

Correspondence address: Mr R. Bevis Cubey, The Ipswich Hospital, Heath Road Wing, Ipswich, Suffolk IP4 5PD.

ophthalmologists in patients on long-term therapy with preparations of eyedrops or eye ointments containing corticosteroids, introduced into the conjunctival sac for such ocular disorders as vernal or allergic conjunctivitis, and uveitis.

There is evidence of genetic predisposition to such a reaction (Armaly, 1965), and the rise in intraocular pressure may be slight or severe. The effect is most marked with dexamethasone, similar with betamethasone, and it has been shown experimentally in human volunteers that the effect of triamcinolone 0·1% four times daily and of prednisolone 0·1% four times daily is similar to that of daily betamethasone (Becker & Hahn, 1964). A similar response has been noted following the systemic administration of corticosteroids, but is much less common. There is usually indirect evidence of obstruction to aqueous outflow from the iridocorneal drainage angle of the eye, and though the underlying mechanism of the obstruction awaits elucidation, mucopolysaccharide accumulation in the trabecular meshwork has been postulated. Symptoms are slight or absent, and although the rise in pressure is usually reversed within 1–2 months of discontinuing the drug, if the pressure has remained elevated for perhaps as little as a few months, permanent ocular damage may result as shown by visual field defects typical of glaucoma and cupping and atrophy of the optic discs (Duke-Elder & Jay, 1969).

In the present case, the theoretical possibility that the ocular response was due to corticosteroid reaching the eye via the circulation, following systemic absorption from the facial skin, seems remote. It is therefore postulated, on strong though circumstantial evidence, that small amounts of the fluocinolone acetonide ointment applied to the skin of the eyelids nightly for 7 years seeped over the lid margins and that sufficient amounts of the active principle were absorbed into the eye in the same way as are topical ocular preparations (predominantly through the cornea) to give rise to glaucoma indistinguishable from typical corticosteroid glaucoma, and intractable in its persistence though without evidence of permanent ocular damage.

Such ocular penetration of corticosteroids applied to the skin of the eyelids could also conceivably parallel the other common ocular side-effects of the same agents deliberately introduced into the conjunctival sac, and induce or exacerbate herpes simplex keratitis or eventually give rise to cataract formation, though the latter is more common following long-term corticosteroid therapy when administered systemically.

There appears to be no other report in the literature of glaucoma presumed to be due to the application to the skin of the eyelids of corticosteroid-containing dermatological preparations.

The purpose of this short report is therefore to alert dermatologists and others to the possibility of silent and potentially irreversible ocular damage resulting from such treatment and to plead for moderation in its use, and the seeking of expert ophthalmological opinion where long-term use is continued.

ACKNOWLEDGMENT

I wish to thank Mr C.Sharp for his advice and permission to report this patient, who is under his care.

REFERENCES

ARMALY, M.F. (1965) Statistical attributes of the steroid hypertensive response in the clinically normal eye. *Investigative Ophthalmology*, **4**, 187.

BECKER, B. & HAHN, K.A. (1964) Topical corticosteroids and heredity in primary open-angle glaucoma. *American Journal of Ophthalmology*, 57, 543.

DUKE-ELDER, SIR W.S. & JAY, B. (1969) Corticosteroid glaucoma. In: *A System of Ophthalmology*, Vol. XI, p. 701. Henry Kimpton, London.

SNEDDON, I.B. (1974) The dangers of the indiscriminate use of topical corticosteroids. *Prescribers' Journal*, **14**, 1.

British Journal of Dermatology (1976) **95**, 209.

Society Proceedings

Dowling club proceedings

27 September 1975, Bristol Royal Infirmary

CHAIRMAN: DR R. P. WARIN

BULLOUS PYODERMA OF LEUKAEMIA. Dr J.L. Burton

A 32-year-old woman

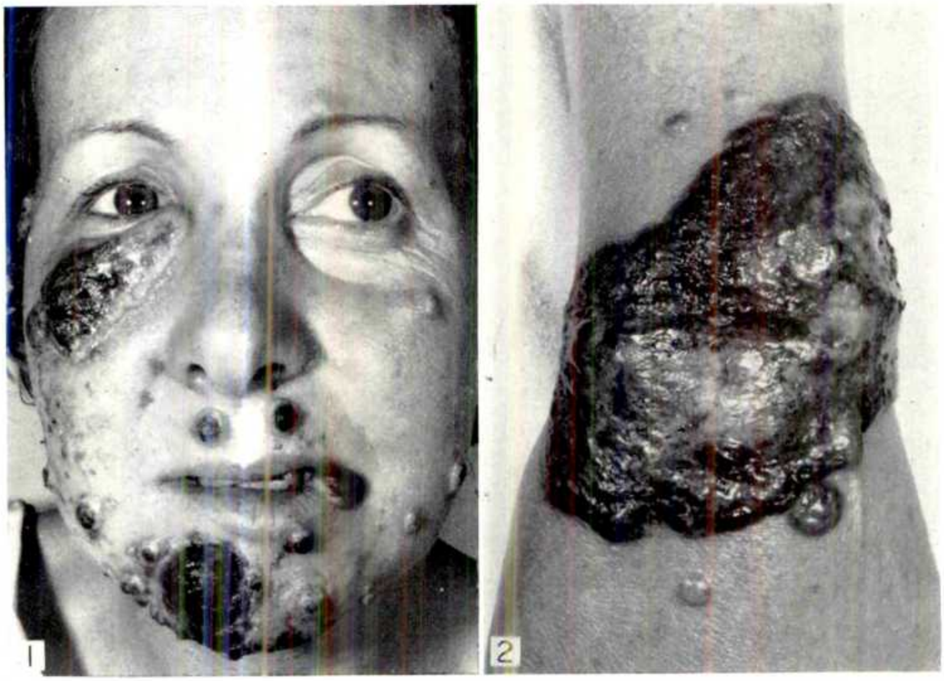
This secretary presented in May 1975 with a 9-day history of steadily enlarging soft purple papules on her forearm and chin. On admission she had a haemoglobin concentration of 9·3 g%, normochromic, normocytic red cells, and a white cell count of 14,500/mm^3, with 82% neutrophils, some showing mild reactive changes. Plasma viscosity was 1·88 cp (normal, less than 1·72 cp). A skin biopsy showed a very dense dermal infiltrate of neutrophils, some capillary thrombosis but no definite vasculitis; a provisional diagnosis of Sweet's neutrophilic dermatosis was made.

At the end of the first week in hospital she developed malaise and became pyrexial, and since new lesions were still developing and the old ones were enlarging rapidly and becoming purulent, she was treated with prednisone 40 mg/daily. In the next few days her white cell count increased to 40,000/mm^3, the cells showing more reactive changes; this was interpreted as a leukaemoid reaction to steroid therapy. She continued to deteriorate, however, and developed hepatosplenomegaly. Sternal marrow and iliac crest aspirations were both 'dry taps', but an iliac trephine biopsy showed a hypercellular marrow with many poorly differentiated blast cells. A diagnosis of acute myelomonocytic leukaemia was made (Dr G. Scott). The haemoglobin continued to fall, and the white cell count rose to 133,000/mm^3. A second skin biopsy showed multilocular intra-epidermal bullae, and the cutaneous lesions were diagnosed as bullous pyoderma gangrenosum (Figs 1 and 2). Direct immunofluorescence of the skin showed fixation of complement in the epidermal basement membrane zone; there was no fixation of IgG, IgM or IgA, and blood complement activity was normal.

Her skin lesions slowly healed with surprisingly little scarring following treatment with prednisone 80 mg daily, and repeated courses of daunorubicin hydrochloride (1·5 mg/kg) and cytosine arabinoside (2·0 mg/kg). Her haematological course was stormy, with many episodes of infection and bleeding, and she eventually died of septicaemia in October, 1975.

COMMENT

Similar cases of atypical bullous pyoderma gangrenosum associated with leukaemia have been reported by Perry & Winkelmann (1972). This case illustrates that the condition can resemble Sweet's



FIGURES 1 and 2. Bullous pyoderma of leukaemia. Each lesion started as a small papule which enlarged steadily and became purulent and necrotic.

neutrophilic dermatosis in the early stages. A similar patient with myeloid leukaemia was shown at the 1972 Summer B.A.D. Meeting (Case 47) and was accepted as Sweet's disease.

REFERENCE

PERRY, H.O. & WINKELMANN, R.K. (1972) Bullous pyoderma and leukaemia. *Archives of Dermatology*, **106**, 901.

MULTIPLE CUTANEOUS MALIGNANCIES IN LEUKAEMIA. Dr R.J. Pye for Dr J.L.Burton

A 53-year-old man

This patient presented in 1971 with malaise and was found to have chronic lymphatic leukaemia with associated hypogammaglobulinaemia. As a result of 3 weeks' treatment with chlorambucil, 10 mg daily, he improved. In May 1974, solar keratoses were noted on the dorsa of both hands. In April 1975, he developed severe bronchopneumonia with marrow failure and was again found to have marked reduction of all immunoglobulins. Once the infection had been controlled, he was given prednisolone 40 mg daily, human immunoglobulins weekly, and chlorambucil 6 mg daily initially, then 2-4 mg daily. The patient remarked that the skin lesions had 'blossomed out' since this treatment started.

On examination he has a Cushingoid facies, steroid acne on his trunk, and the following skin tumours: (i) Keratoacanthoma on the right side of his neck; (ii) Basal cell epithelioma on the right side of his nose; (iii) Squamous cell carcinoma on the right side of his forehead; (iv) Squamous cell carcinoma on the dorsum of his left hand.

He also has multiple solar keratoses on his face and the dorsa of his hands.

COMMENT

Walder, Robertson & Jeremy (1971) examined fifty-one immunosuppressed transplant patients, and found a total of 19 skin tumours in seven of them. Two of the patients with solar keratoses had developed multiple squamous cell carcinomas.

We feel that our patient's multiple solar keratoses may have undergone malignant transformation as a result of his idiopathic and iatrogenic immunosuppression. We suggest that patients requiring treatment that may cause immunosuppression should have any solar keratoses treated with topical 5-fluorouracil cream in an attempt to prevent later malignant change.

REFERENCE

Walder, B.K., Robertson, M.R. & Jeremy, D. (1971) Skin cancer and immunosuppression. *Lancet*, **ii**, 1282.

MULTIPLE BASAL CELL CARCINOMATA OCCURRING IN AREAS PREVIOUSLY EXPOSED TO EXCESSIVE AMOUNTS OF DIAGNOSTIC X-RAYS IN THREE PATIENTS. Dr C.N. Matthews for Dr R.R.M.Harman and Dr R.P.Warin

Case 1. A 45-year-old man

A bony abnormality of the right side of his skull had been first noticed in early childhood; it was thought to be fibrous dysplasia or multiple osteomata. Between the ages of 11 and 18 years he had approximately 200 skull X-rays per year. Then, between the ages of 20 and 40 years he had usually had 4 skull X-rays each year, and since then one X-ray yearly. The total dose of irradiation has been calculated at 3,530 rads ±50%.*

There has been no other known exposure to carcinogenic agents. Since 1973, six histologically proven basal cell carcinomata (BCC's) have been curetted from the right side of his face and neck.

COMMENT

It is tempting to link the bony abnormality with the later development of multiple BCC's in the overlying, previously normal skin; but this is neither Gorlin's nor any other previously described syndrome, and it strongly suggests that the X-irradiation has been an important aetiological factor.

Case 2. A 56-year-old woman

Between 1944 and 1949 inclusive, this patient had artificial pneumothorax treatment for pulmonary tuberculosis. This necessitated frequent chest screenings and subsequent full size chest X-rays. From the available evidence it has been calculated that she received a total dose of 1,253 rads ±50%.*

She admits to having received herbal medicines in childhood. Since 1973, she has developed multiple, histologically proven BCC's over her upper back and neck.

COMMENT

The localization of her BCC's, and the lack of keratoses and skin neoplasia elsewhere, suggest that the X-rays have been aetiologically relevant.

---

* Calculations made by Mr D.Gifford, Area Radiological Protection Adviser, Bristol.

Case 3. A 35-year-old man

For almost 40 years, this patient has suffered from arthritis of both hips, and has had X-rays of the pelvis and hips on many occasions. It is not possible to estimate the total dose because of lack of records.

He also claims to have had monthly sun-lamp treatment to his hips for 20 years, and in 1948–1949 he had radiotherapy to the upper dorsal spine. There is no history of arsenic ingestion. Since 1973, he has had three BCC's in his groins.

COMMENT

It is possible that the unusual localization of this man's three BCC's is related either to the X-ray exposure, to the sun-lamp therapy or to both. Although previous diagnostic X-irradiation is not normally considered as a cause of BCC's developing in previously normal skin, Ingram (1964) referred to a radiologist with multiple BCC's of the trunk who, for many years, had taken chest X-rays of himself to test his X-ray machine.

It is suggested that, in these cases, diagnostic X-rays have been aetiologically relevant in the subsequent development of multiple BCC's.

REFERENCE

INGRAM, J. (1964) *British Journal of Dermatology*, **76**, 294.

CUTIS GYRATA OF THE FACE WITH BENIGN PARAPROTEINAEMIA. DR R.R.M. Harman

A 66-year-old man

History. Thickening of the nose and facial skin has been present since boyhood, and has worsened with age. It now closes the left eye with oedematous lids which have been affected by cellulitis. He has become increasingly asthenic over the last 4 years. In the past, he has had a cataract in his left eye and renal colic from a right pelvic kidney.

Family history. Both his father and one sister have all their lives had similar facial thickening.

Examination. There is cutis gyrata of the face, particularly striking on the forehead, with some seborrhoea and acne lesions. The oedematous left upper lid overhangs the eye. The scalp and hair are normal, there is acne scarring on the trunk, the fingers and toes show no acromegalic changes, and the palms are normal.

The blood pressure is 170/95 mmHg, the heart is enlarged, and on auscultation there are mitral and aortic murmurs. The liver is palpable 3 cm below the right costal margin, but there is no lymphadenopathy and the respiratory system is clinically normal.

Investigations

Haematological. Haemoglobin 11·3 g %, MCV 89 μm^3, WBC 7,500/mm^3 with a normal differential count, and a blood film showing rouleaux formation. Plasma viscosity 2·78. Bone marrow normal.

Urinalysis normal; no Bence Jones proteinuria.

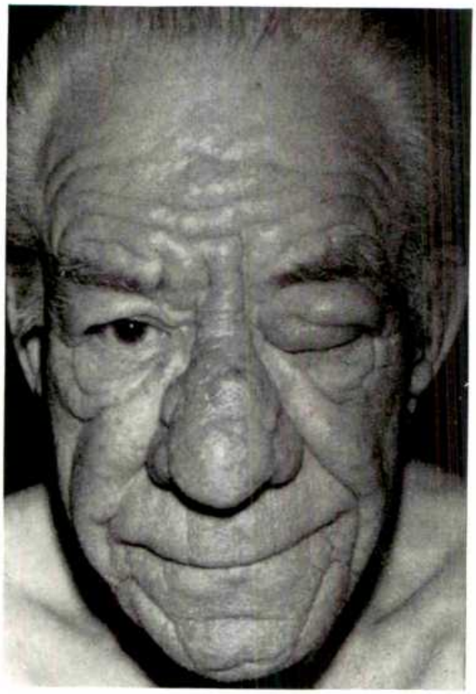


FIGURE 3. Cutis gyrata of the face. The odematous left upper lid overhangs the eye.

Biochemical. Blood urea 38 mg%; liver function tests normal. Thyroid function tests, serum growth hormone level and glucose tolerance test all normal.

Radiological. Chest X-ray and a skeletal survey, including coned views of the pituitary fossa, normal and no evidence of myelomatosis.

Electrocardiography. P–R interval prolonged, changes consistent with left ventricular hypertrophy, and T waves inverted in leads V_4, V_5 and V_6.

Histology of forehead skin. Sections show a piece of hair-bearing skin with prominent sebaceous glands. There is no folding, and the epidermis shows an area of thinning with flattening of the rete ridges. The upper dermis contains large areas of degenerate collagen. A mild chronic inflammatory infiltrate is also seen.

COMMENT

Cutis gyrata usually involves the scalp (verticis), and a family history is unusual. In this case there is no association with developmental syndromes or microcephalic idiocy. The association with benign paraproteinaemia is probably fortuitous.

MOLLUSCUM CONTAGIOSUM WITH GIANT LESIONS. DR R.D.G.PEACHEY

A 60-year-old woman

History. In 1971 she developed scaling and erythema in the scalp and flexures, which was diagnosed as seborrhoeic eczema. In 1974 she was admitted to hospital with a diagnosis of seborrhoeic dermatitis, but had a number of 'prurigo-like' nodules on the trunk and limbs, and on her soles.

In the following months, widespread papular and nodular lesions appeared, which were diagnosed, clinically, as molluscum contagiosum.

Examination. There is widespread mild eczema with hundreds of scattered lesions of molluscum contagiosum which, in places, are confluent—producing plaques and 'giant' lesions. Fig. 4 shows a large plaque behind the left ear.

Investigations. Biopsies of a lesion on the abdomen and of the plaque behind the left ear showed histology of molluscum contagiosum. All others investigations done, including immunoglobulin estimations, were normal.

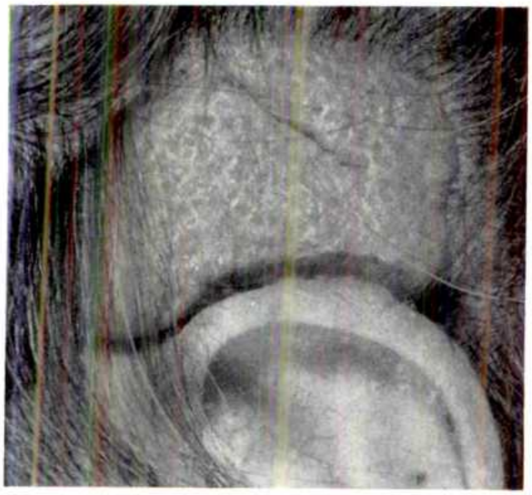


FIGURE 4. Large plaque of molluscum contagiosum lesions behind left ear.

Treatment. She had so many lesions of molluscum contagiosum, that local treatment was of little benefit. A trial of adenosine arabinoside in DMSO—applied topically under polythene occlusion—did not have any effect. Oral administration of methisazone—3g thrice daily for 3 days—was also unhelpful.

COMMENT

Confluent lesions of molluscum contagiosum producing plaque formation may be easily misdiagnosed. Although we have been unable to find any underlying abnormality, it seems possible that this patient may have an immunological defect to account for her profuse and exaggerated lesions.

British Journal of Dermatology (1976) **95**, 215.

Book Reviews

Morphogenesis of Skin. PHILIPPE SENGEL (1976) Cambridge University Press. Pp. viii+277, illustrated. Price £14.00.

In this splendid little book Professor Sengel has brought together and synthesized the very considerable fund of knowledge at present in our possession concerning the embryonic and post-natal development of skin. Most of this information has hitherto been scattered amongst a wide range of journals and symposium proceedings not normally read by dermatologists. It is indeed a boon to have it now collected into book form by an author who has not only made great contributions to the subject himself but lucidly and readably expounds the work of others. At the same time he equally clearly points out the vast field of ignorance which remains a challenge to us all.

Anyone having a genuine interest in disease of the skin, whether from a purely clinical or from a research viewpoint, will enjoy and benefit from reading this account of how the tissue they love comes into existence. And after all, much post-natal skin disease does date back to faulty embryonic development.

It is to be hoped sincerely that the rather high price will not dissuade too many dermatologists from reading it. It should certainly be in every dermatological library and form part of the 'background reading' of all trainees.

I.W.WHIMSTER

The Vulva. CONSTANCE MARJORIE RIDLEY (1975) London: W.B.Saunders. Pp. 299, illustrated (B/W).

Specialization in medicine has many triumphs to its credit, but is not without its shortcomings, particularly when specialities become very circumscribed, sometimes leading to an arrogant, and occasionally spurious, air of omniscience. Happily, artificial separation of different but overlapping branches of medicine has tended in recent years to give way to joint study by two or more specialities. The well known humility of dermatologists has not always been to their advantage, or to their subject, which can justly claim priority in the delineation of disorders such as lupus erythematosus, sarcoidosis, and more recently, the skin markers of abnormal metabolism and systemic neoplasia, to take just a few examples. Unfortunately, adequate joint studies between gynaecologists and dermatologists on the very difficult problems of vulval skin disorders are still conspicuously lacking, and the result readily apparent in most textbooks of gynaecology.

Dr Ridley, a dermatologist of the highest distinction, has had the good fortune over many years to make a joint study of vulval skin disorders with her gynaecological colleagues, and her book shows that she has taken every advantage of this opportunity. Scholarly, erudite, and constructively critical, she has succeeded brilliantly in combining her own great experience with the observations of most other authors on vulval physiology and pathology. Detailed references abound without destroying continuity. All this has been achieved in a small volume, beautifully produced, with photographs of a high standard. Dr Ridley merits the warmest congratulations and gratitude for her book, which will rapidly come to be recognized as the standard work on vulval skin disorders, indispensable to all practising gynaecologists and dermatologists, and as a reference book in all medical libraries.

H.J.WALLACE

Patch Testing. S.FREGERT and H.J.BANDMANN (1975) Berlin: Springer. Pp. 78, 3 illustrations, 17 tables, soft cover. Price $7.50.

Patch Testing Guidelines. K.E.MALTEN, W.G.VAN KETEL and J.P.NATER (1976) Nijmegen: Dekker and Van de Vegt. Pp. 135, 5 illustrations. Price Dfl. 29.50.

After a long period of comparative neglect, contact dermatitis has come in from the cold, burgeoning books in its wake. The vast number of allergens now present in the natural, synthetic and industrial environment, and the difficulties inherent in application and interpretation of the *in vivo* techniques of patch testing are reason enough for the widest possible dissemination of information for the use of the dermatologist, the patch test nurse (or technician) and the patient himself. Three such books—all in soft covers and relatively cheap—have appeared on the British market in the last 2 years. The first was Fregert's *Manual of Contact Dermatitis*, already reviewed in the journal (*British Journal of Dermatology*, **93**, 235). This has been so well received that all others must be judged in relation to it. The German edition of the Fregert–Bandmann manual (also published on behalf of the International Contact Dermatitis Research Group) has also been enthusiastically reviewed (*British Journal of Dermatology*, **90**, 590). The English edition can now be welcomed. Though slightly shorter than the Fregert, it has almost the same amount of text and substantially more detail in regard to the supplier, concentration and vehicle of over 120 allergens and the sources of possible contact.

One of the authors of *Patch Testing Guidelines* is also a member of the Group and his particular views and interests are presented in a more personal manner—for instance his use of the 'silver' (square) patch test. The text is rather more concentrated and his particular attention is given to domestic and industrial sources of all the common allergens and to instruction and advice to the patient. This includes many practical details and a choice collection of the patch testers stamp album of curiosities (e.g. tetramethylthiuramsulphide is present in meatballs in automat slot machines). This is a stimulating book to read but, though it lacks the easily-found tables of concentrations given in the other two, it has a most useful list of 255 references. The translation is adequate but there are a few odd examples of English usage (limited is 'Ltd', not 'Lim', etc.; neomycin drops the final e); but these can easily be corrected in the next edition.

It may seem invidious for another member of the ICDRG to be asked to review these particular books. But I think I can reflect the views of my colleagues working in this field with the usual tight budget. I have also asked the views of my nurses and colleagues working in our Contact clinic. These are the people to whom all three books are addressed. I would not wish, personally, to be without any of them, since I find them complementary. But if one is limited to two, we all felt that one of the Fregert books should be supplemented by the 'Guidelines'. Thus equipped, any dermatologist should be able to justify his interpretation of the results of his patch testing not only to the patient but also to a medical tribunal or Court of Law.

D.S.WILKINSON

Rational Diagnosis and Treatment. H.R.WULFF (1976) Oxford: Blackwell Scientific Publications. Pp. 182. Price £3.75.

This is an important book which all thinking clinicians should read. A casual glance through the pages suggests that it is a dreary treatise for logicians, but this impression is wrong. It is in fact concerned with practical problems of patient management and it is written in a readable and lucid style. In essence the book is about a new subject called 'clinical decision theory' which encourages doctors to reassess critically their interpretation of clinical and laboratory data, their use of diagnostic tests and their choice of treatment. The jargon of this new discipline is somewhat forbidding ('nosography', 'threshold of iatrotropy', 'minimax loss principle', etc.), but the terms are clearly defined and should present little difficulty to a profession which appears to cope adequately with 'pityriasis lichenoides acuta' and 'cholinergic dermographism'.

The rational approach to the practice of medicine has of course already been eloquently extolled by Richard Asher, J.G.Scadding and others, but Wulff has extended the process of ratiocination to include the use of mathematics to measure the degree of uncertainty which is inherent in most clinical decisions. The reasons for taking a particular decision thus become explicit and subject to analysis, and clinical reasoning should in theory become as rigorous and logical as that in any other scientific discipline. The technique is used intuitively by good clinicians to a certain extent, although like the man who found he spoke prose, the clinician may be pleasantly surprised to learn that he thinks in mathematical sets, using symbolic logic which is conveniently expressed in the notation of Boolean algebra. Those who fear that this mathematical approach will remove the art from medicine are reminded by Wulff that it is the controlled trial which has emphasized the therapeutic importance of the doctor–patient relationship.

The book is packed with thought-provoking observations on topics such as the definitions of diseases, the principles underlying the evaluation of data, and the logical use of different ranges of normal values to establish or exclude a particular diagnosis. The occasional contentious statement, such as the claim that an investigation must be considered unethical if it can be predicted that the result will not benefit the patient, only adds to the intellectual invigoration.

Wulff has the admirable gift of elucidating the ideas behind statistics without fussing about the mathematical details, and his comments on the interpretation of the statistics of therapeutic trials will be valuable to many clinicians. Many other aspects of clinical measurement are succinctly highlighted including problems of bias, reproducibility, random variation and the difference between imprecision and inaccuracy. Wulff champions the use of 'soft' data in clinical research, he demolishes the dogma that multiple medical measurements form a Gaussian distribution until proved otherwise, and he exposes the trick of producing statistically significant results simply by performing a multiplicity of comparisons. And for the literati who remain adamant that numeration is noxious, the book is liberally spiced with fascinating historical vignettes. I'll bet you didn't know that, according to Pliny, enemata originated in ancient Egypt because the ibis used its curved bill to give itself rectal infusions of Nile water.

This book should at least help to encourage clarity of thought, and it may even inculcate in a few readers that admirably dispassionate approach which is illustrated by Wulff's story of the statistician who on being asked 'How is your wife?' replied 'Compared to whom?'

J.L.BURTON

Veins and their Control. JOHN T.SHEPHERD and PAUL M.VANHOUTTE (1975) London: W.B.Saunders. Pp. 269, illustrated.

This book is concerned with the veins of the splanchnic area, muscle and skin. Concerning the latter it is the large capacity veins rather than the dermal venules that are discussed. Much of the book is devoted to the pharmacology of smooth muscle with a special interest in activation by calcium as well as in metabolic factors and the ionic changes influencing venous reactivity.

There are brief chapters on physical factors such as the influence of gravity, shock or congestive heart failure on venous function. This is a succinct and well referenced account of how veins function which is probably of value to the physiologist but not of great interest to those concerned with disease of the skin. Skin physiologists are usually particularly interested in the role played by venules while clinicians are concerned with disorders of the lower leg. This book does not provide more than a few paragraphs to explain stasis syndromes and varicose veins.

T.J.RYAN

Dermatologie. W.BRAUN (1975) Berlin: Veb. Verlag Volk und Gesundheit. Pp. 408, 101 illustrations, 12 tables. Price DM 19.60.

Dr Waltraud Braun is Director of the Dermatological Department of the Martin Luther University, Halle, Wittenberg, and contributors come from Dresden, Leipzig, Jena, Rostock, as well as her own department. This book is thus a product of the German Democratic Republic.

The volume, about the size of a 'year book', comprises nearly 400 pages and is most comprehensive. There is a large section on 'Introduction to Diseases of the Skin', which deals with the anatomy, physiology and biochemistry of the skin in some detail. Instructions on the taking of a history, deductions from the distribution of various types of eruptions are fully illustrated with generous, excellent black and white photographs. Venereology still forms a very large part of a continental dermatologists work, and so there is considerable space given to venereological matters. Each contribution is by an expert in his field, and

British Journal of Dermatology (1976) **95**, 219.

Review

Treatment of obesity

APPETITE SUPPRESSANT DRUGS AND INTESTINAL BY-PASS SURGERY

Obesity is a disorder in which there is an excess of adipose tissue mass that is the result of an imbalance between energy intake and expenditure. The excess of calories results in the deposition of fat. Many factors are involved in obesity, the prevalence of which is increasing in the developed countries of the world and where it is now the commonest form of malnutrition (Shank, 1965). One possible causative factor is an excessive calorie intake in early life. Infantile over-nutrition in artificially fed infants in the Sheffield region was shown to be very common especially in the first 6 weeks of life (Taitz, 1971). The early rapid weight gain in these infants is considered to influence the total number of adipose cells (Beaton, 1967) so that the fat baby is quite likely to be a fat adult. Lloyd, Wolff & Whelan (1961) found that 80% of obese children remained obese in adult life. Though the majority of fat patients over eat, it is also apparent that they are relatively inactive (Chirico & Stunkard, 1960). Increased exercise alone is an effective way of losing weight (Gwinup, 1975). It is difficult to assess accurately the risks of being obese, but data from the life insurance companies clearly indicate that those who are overweight are more likely to die prematurely, especially those who are grossly obese. There is also an increased morbidity from cardiovascular diseases. These risks can be lessened by a reduction of weight.

At present, there is no easy or quick treatment for the obese. Most patients will require a complete re-education as to their diet with restriction of their intake of food and a permanent control of the number of calories they eat.

In this review, two controversial forms of therapy in the management of the obese patient are discussed—the use of appetite suppressant drugs and intestinal by-pass surgery. It must be emphasized that there is no substitute for will-power in achieving weight reduction (British National Formulary, 1974–76). Motivation is all important, with group therapy usually more effective than when the patient goes it alone. Societies such as 'Weight Watchers' have in general better results than hospital clinics and often succeed when doctors and dietitians have failed.

Appetite suppressant drugs

In 1973 the N.H.S. costs in England for drugs promoted for the treatment of obesity was £2,478,926. This considerable sum indicates the demand from the public for appetite suppressant drugs and that in our society a pill is preferred to self denial. The aim of these drugs is to help the patient to adhere to a reduction in their calorie intake and most of them are only useful over a short term of therapy. At present there is no completely effective and safe appetite suppressant drug that can be given for a prolonged period (Anderson, 1974).

Fenfluramine hydrochloride ('Ponderax', 'Ponderax PA'). This drug, which is chemically related to amphetamine, is widely used as an appetite suppressant. Its action is not fully understood (Sapieka, 1972; Garrow, Belton & Daniels, 1972), but in clinical trials it was shown to have a central anorectic

Reprint requests: Dr S.S.Bleehen, Department of Dermatology, Hallamshire Hospital, Sheffield S10 2JF.

effect (Munro, Seaton & Duncan, 1966; Spence & Medvei, 1966). In therapeutic doses it has a depressant action on the cerebral cortex as it affects the appetite regulating centre in the hypothalmus. It is also believed that fenfluramine has a so-called 'glycolyptic' action and that ingested carbohydrate is diverted away from adipose tissue by increasing glucose uptake in muscle (Butterfield & Whichelow, 1968). The mobilization of fat from its depots is also enhanced by this drug (Pawan, 1969). These metabolic actions of fenfluramine have been questioned (Garrow *et al.*, 1972) and it is likely that the weight loss is solely due to its central anorectic effect. The dose of 'Ponderax' is initially 20 mg twice daily and then increased to 40 mg twice daily and 'Ponderax PA' 60 mg once or twice daily. Side effects are common, especially nausea, dizziness, depression, drowsiness and diarrhoea. It should not be given with or within 3 weeks of mono-amine oxidase inhibitors or during pregnancy. Sudden withdrawal of the drug may result in depression (Steel & Briggs, 1972) and it is well recognized that some patients become dependent on it.

Diethylpropion hydrochloride ('Apisate' and 'Tenuate Dospan'). This drug is chemically related to amphetamine and has been shown to have an anorectic effect (Hadden & Lucey, 1961). Like fenfluramine it should not be given to those who are emotionally unstable since drug abuse and addiction are not uncommon. The usual dose is 75 mg once daily, usually in the morning. The drug is contraindicated for those patients on mono-amine oxidase inhibitors. Side effects are not infrequent and include a dry mouth, nausea, constipation and dizziness.

Phentermine ('Duromine' and 'Ionamin'). This is also related to amphetamine and it is used in the form of a resin complex with a sustained action. It is taken once daily in a dose of 15 mg usually before breakfast and is given in intermittent courses. Like the other appetite suppressant drugs, it is most effective over a short term of therapy.

Amphetamines ('Durophet' and 'Durophet M'). These are controlled under the Misuse of Drugs Act 1971. Both are a mixture of laevo and dextro-amphetamine in ratio 1:3 as resin complexes and Durophet M contains methaqualone. There is the real danger of drug abuse with habituation and dependency (Edison, 1971). While amphetamines undoubtedly suppress the appetite, they should be used with caution. They should not be prescribed for patients with hypertension or cardiovascular disease or to those who are on or who have been recently taking mono-amine oxidase inhibitors.

Phenbutrazate hydrochloride and phenmetrazine theoclate ('Filon'). This preparation is also controlled under the Misuse of Drugs Act and there is a risk, especially in the emotionally unstable, of drug dependency. The usual dose is one tablet 2 or 3 times daily before meals.

Mazindol ('Teronac'). This is a relatively new anorectic drug with a chemical structure unrelated to the amphetamines. Short term studies have indicated that it will induce weight loss approximating that produced by d-amphetamine both in animals and in man and without producing a drug dependency (Hadler, 1972; Defelice, Chaykin & Cohen, 1973).

In company with other anorectic agents, such as fenfluramine, claims have been made for a lipid lowering effect in hyperlipidaemic individuals even in the absence of weight loss. The effect on cholesterol and triglyceride levels is similar and an improvement of abnormal glucose tolerance tests has been observed (Dolocek and Zavada, 1973). These authors could not show any change in thyroid or liver function. Side effects of Mazindol are largely insomnia, dry mouth, dizziness (often postural), together with a lesser incidence of dysuria and constipation (Hadler, 1972; Defelice *et al.*, 1973; Dolocek & Zavada, 1973), but on the whole the drug is well tolerated. However, the drug invariably produced an increased pulse rate and a rise in the supine blood pressure. The postural drop in systolic blood pressure is less than usual, but the pulse pressure and pulse rate on standing are both increased (Hadler, 1972; Edwards, 1974). The clinical significance of these changes is unknown but obviously one should be cautious in prescribing this drug for patients with heart disease, thyrotoxicosis, hypertension or other conditions likely to embarrass the cardiovascular system. Mazindol should be given in a dose of 2 mg once daily in the morning prior to lunch. The drug is expensive but it may be of use over a short term as an adjunct to dietary treatment though more clinical experience is required to evaluate its effectiveness.

Bulk preparations

Both methylcellulose and Guar gum can be prescribed and there are various prescribable proprietary preparations that contain these, either in the form of tablets or as granules. In addition there are a variety of non-prescribable slimming foods that contain these preparations. These are taken with water just prior to meals and, after swelling in the stomach, produce a sense of fullness. They are harmless, but are also ineffective (Munro, 1973).

Intestinal by-pass operations

These operations are only undertaken as a drastic attempt to treat the very obese patient when all other measures have failed. The risks of these operations are quite considerable and have to be balanced against the risk of the patient remaining severely obese. The jejuno-ileal shunt is the operation now preferred to the jejuno-colic by-pass originally used. Experience has shown that the jejuno-ileal by-pass is safer and that the jejuno-colic operation should not be carried out (Payne & DeWind, 1969). However, even with the jejuno-ileal shunt, there is still an appreciable risk of subsequent hepatic damage, cirrhosis and death from liver failure (McGill *et al.*, 1972; Mangla *et al.*, 1974). The risks are difficult to assess accurately since a number of patients have had their by-pass operations taken down, the hepatic changes often being reversed when normal intestinal continuity is restored. Perhaps better pre-operative screening of the patients and careful postoperative follow-up may reduce these risks. One further feature is that there is a surprising psychosocial improvement in those obese patients who have been treated with by-pass surgery and who, as a result, have lost several stones in weight (Solow, Silberfarb & Swift, 1974).

Academic Division of Medicine,
and
Department of Surgery,
University of Sheffield

S.S.Bleehen
I.R.Edwards
R.G.Clark

REFERENCES

Anderson, J. (1974) Drugs and appetite. *The Practitioner*, **212**, 536.

Beaton, J.R. (1967) Energy balance and obesity. *Canadian Journal of Public Health*, **58**, 479.

Butterfield, W.J.H. & Whichelow, M.J. (1968) Fenfluramine and muscle glucose uptake in man. *Lancet*, **ii**, 109.

Chirico, A.M. & Stunkard, A.J. (1960) Physical activity and human obesity. *New England Journal of Medicine*, **263**, 935.

Defelice, D.A., Chaykin, L.B. & Cohen, A. (1973) Double-blind clinical evaluation of mazindol, dextro amphetamine and placebo in the treatment of exogenous obesity. *Current Therapeutic Research*, **15**, 358.

Dolocek, R. & Zavada, M. (1973) Hypolipidaemic effect of a new anorectic—AN 448. *Časopis Lékařů Českých*, **112**, 144.

Edison, G.R. (1971) Amphetamines: A dangerous illusion. *Annals of Internal Medicine*, **74**, 605.

Edwards, I.R. (1974) Unpublished observations.

GARROW, J.S., BELTON, E.A. & DANIELS, A. (1972) A controlled investigation of the glycolyptic action of fenfluramine. *Lancet*, **ii**, 559.

GWINUP, G. (1975) Effect of exercise alone on the weight of obese women. *Archives of Internal Medicine*, **135**, 676.

HADDEN, D.R. & LUCEY, C. (1961) Diethylpropion in the treatment of obesity. A cross-over trial of a long-acting preparation. *Ulster Medical Journal*, **30**, 1109.

HADLER, A.J. (1972) Mazindol: a new non-amphetamine anorexogenic agent. *Journal of Clinical Pharmacology*, **12**, 453.

LLOYD, J.K., WOLFF, O.H. & WHELAN, W.S. (1961) Childhood obesity: a long term study of height and weight. *British Medical Journal*, **ii**, 145.

MANGLA, J.C., HOY, W., KIM, Y. & CHOPEK, M. (1974) Cirrhosis and death after jejuno-ileal shunt for obesity. *American Journal of Digestive Diseases*, **19**, 759.

MCGILL, D.B., HUMPHREYS, S.R., BAGGENSTOSS, A.H. & DICKSON, E.R. (1972) Cirrhosis and death after jejuno-ileal shunt. *Gastroenterology*, **63**, 872.

MUNRO, J.F. (1973) The management of obesity. *British Journal of Hospital Medicine*, **10**, 8.

MUNRO, J.F., SEATON, D.A. & DUNCAN, L.J.P. (1966) Treatment of refractory obesity with fenfluramine. *British Medical Journal*, **ii**, 624.

PAWAN, G.L.S. (1969) Effects of fenfluramine on blood lipids in man. *Lancet*, **i**, 498.

PAYNE, J.H. & DEWIND, L.T. (1969) Surgical treatment of obesity. *American Journal of Surgery*, **118**, 141.

SAPIEKA, N. (1972) Anti-obesity action of fenfluramine. *The Practitioner*, **208**, 660.

SHANK, R.E. (1965) In: *Preventive Medicine for the Doctor in his Community* (Ed. by H.R.Leavell and E.G.Clark), p. 171. McGraw-Hill, New York.

SOLOW, C., SILBERFARB, P.M. & SWIFT, K. (1974) Psychosocial effects of intestinal by-pass surgery for severe obesity. *New England Journal of Medicine*, **290**, 300.

SPENCE, A.W. & MEDVEI, V.C. (1966) Fenfluramine in the treatment of obesity. *British Journal of Clinical Practice*, **20**, 643.

STEEL, J.M. & BRIGGS, M. (1972) Withdrawal depression in obese patients after fenfluramine treatment. *British Medical Journal*, **iii**, 26.

TAITZ, L.S. (1971) Infantile overnutrition among artificially fed infants in the Sheffield region. *British Medical Journal*, **i**, 315.

British Journal of Dermatology (1976) **95**, 223.

News and Notices

Willan Library

The Willan Library, the property of the British Association of Dermatologists, is housed in the Willan Room of the Royal College of Physicians of London. It consists of a rapidly growing collection of books and journals illustrating the history of dermatology up to the present day. Readers of this Journal may have books or journals for which they no longer have any use and which they are prepared to present to the Library. All such gifts will be greatly appreciated.

The Librarian hopes to expand the interesting collection of offprints ('reprints'). Authors are invited to send to the Librarian a signed offprint of each of their papers, past and future. Offprints are indexed under authors and subjects.

Access to the Library can be provided to those engaged in research in the history of dermatology, with the approval of the Executive Committee of the Association. The Willan Librarian will be glad to receive, and will attempt to answer, any enquiries concerning the history of dermatology.

Gifts to the Library and all correspondence concerning Library affairs should be addressed to Dr Arthur Rook, Honorary Willan Librarian, Department of Dermatology, Addenbrooke's Hospital, Cambridge.

The Royal College of Physicians

The following Dermatologists have recently been elected to the Fellowship of the Royal College of Physicians, London:

Dr D.C.G. Bett; Dr L. Fry; Dr F. A. Ive; Dr L. A. M. B. Musso; Dr B. Portnoy; Dr E. A. Ryan; Dr M. A. Smith; Dr M. M. Walshe.

APPOINTMENTS

Stobhill Hospital, Glasgow

Dr Rebecca Cochran has been appointed consultant dermatologist at Stobhill Hospital, Glasgow. Dr Cochran qualified M.B., Ch.B. (Glasgow) in 1964 and obtained her M.R.C.P. (U.K.) in January 1971. She has been a Collegiate Member of the Royal College of Physicians and Surgeons of Glasgow since 1972. Dr Cochran began her career in dermatology as Senior House Officer in the Dermatology Department at the Western Infirmary, Glasgow, from 1966 to 67, following which she returned to the Victoria Infirmary, Glasgow, to enlarge her experience in general medicine. In 1972 she returned to the Western Infirmary Dermatology Department as Registrar, and in May 1973, was appointed to her present post as Lecturer in the University Department with Honorary Senior Registrar status at the Western Infirmary. In the latter post she has been mainly responsible for the organisation of the undergraduate teaching of the department. Her particular research interest is the clinical management of atopic dermatitis, and she has recently made a study visit to Scandinavia in connection with this.

Staffordshire Area Health Authority

Dr John David Boxley has been appointed Consultant Dermatologist to the North Staffs Hospital Centre, Stoke-on-Trent and the Stafford General Infirmary, Stafford. Dr Boxley graduated M.B., B.Chir. (Cambridge) in 1968 and became M.R.C.P. (U.K.) 1971. He did his undergraduate training at Guy's Hospital (London). He was Senior House Officer and Registrar in General Medicine and Dermatology at High Wycombe, Buckinghamshire, then moving to St Thomas' Hospital, London, as a Registrar in Dermatology, with a Clinical Assistantship at St John's Hospital for Diseases of the Skin. He was Wellcome Research Fellow in the Department of Immunology at the Institute of Dermatology, St John's Hospital in 1974 and later became Senior Registrar in Dermatology in the North Staffs Hospital Centre, Stoke-on-Trent. Interests include immunologically-mediated skin diseases and the epidemiology of dermatological problems in various industries.

St John's Hospital for Diseases of the Skin

Dr Andrew P.Warin has been appointed Consultant Dermatologist to St John's Hospital for Diseases of the Skin, London. Dr Warin qualified MB, BS, May, 1967, after training at Guy's Hospital Medical School. His junior house jobs were also at Guy's but in 1971 he became Registrar in Dermatology at St Thomas's Hospital, later transferring to the London Hospital and finally to St John's Hospital as Senior Registrar. He then spent a year in Shiraz, Iran, gaining considerable experience in the teaching of dermatology and having a very considerable interest in Leishmaniasis. He has had many papers to his credit and has shown particular interest in the use of Methotrexate in Psoriasis in the Systemic Manifestations of Gonorrhoea and in a Glucagonoma Syndrome. In his new post at St John's he will be involved in pharmacological experiments as well as clinical duties.

International Society of Tropical Dermatology Symposium on Climate and Ecology in Skin Diseases at Las Palmas, Canary Islands

November 1st–3rd 1976

1 November 4.00 p.m.

Introduction: A.Rook. Role of Climate and Ecology in Skin Diseases: O.Canizares. Ozone and the skin: J.L.Burton. Chloracne: K.D.Crow. Mycombacterium Ulcerans Infection: M.Hutt. Skin Changes in Albinos: A.Okoro. Pellagra: G.Lombolt.

2 November

Symposium on photobiology: Chairman: L.Harber

Speakers include W.Frain-Bell, C.D.Calnan, J.Pinol Aguadé, R.H.Meara, F.Urbach, L.Harber, K.Wolff, J.M.Mascaro.

3 November

Workshop on the Treatment of Psoriasis: Chairman: F.Urbach.

Speakers include F.Sagher, K.Wolff, L.Harber, W.Frain-Bell, J.Gomez Orbaneja, A.C.Gyorko-Gyorkos, D.S.Wilkinson, Professor Hartung.

Saturday 6 November 1976 (in Madrid) 9.00 a.m.

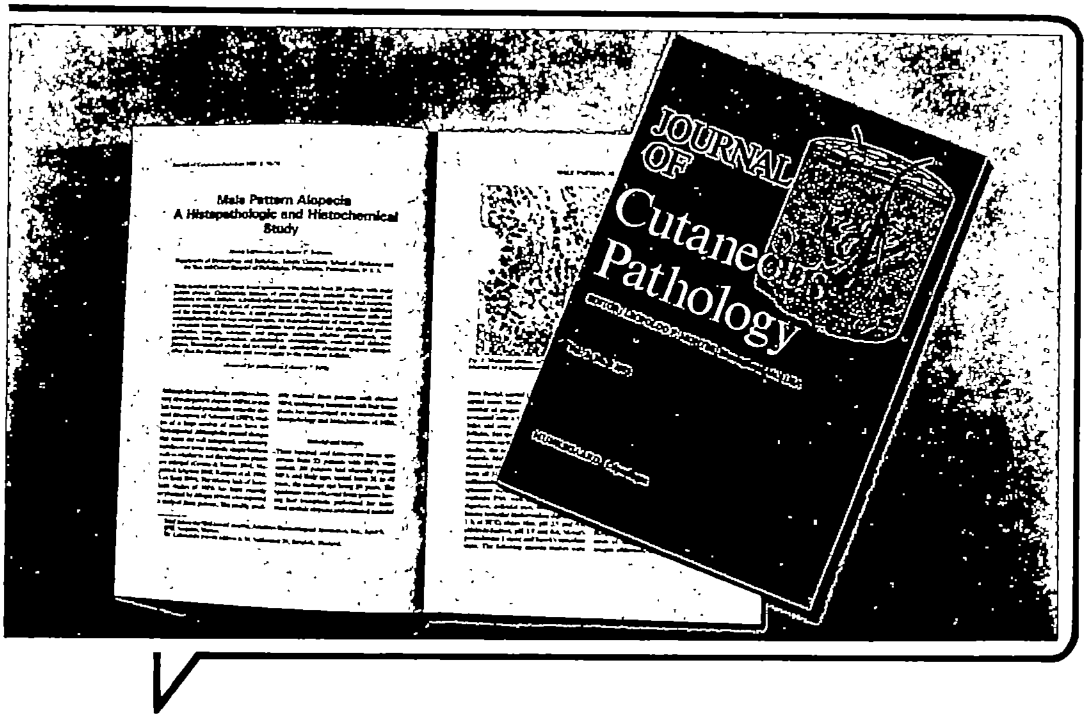
Clinical Presentation of Patients and Discussion: Hospital Clinico, University City: J.Gomez Orbaneja and Staff.

Those wishing to attend, or submit titles for free communications, should contact: Dr Alejandro de Gyorko-Gyorkos, Villa-Hungria, Las Perreras 63, Las Palmas, Canary Islands. Or information may be obtained from Dr R.Harman, Bristol Royal Infirmary, Bristol.

Post-Graduate Medical School, University of Cambridge

The annual introductory course on 'Biology of Skin' will be held at Fitzwilliam College from 7 to 10 December 1976. Enquiries and applications should be sent to: The Secretary, Cambridge University Medical School, Hills Road, Cambridge.

Dermatology



Journal of Cutaneous Pathology

Editor: Leopoldo F. Montes, Birmingham, Alabama

Journal of Cutaneous Pathology publishes manuscripts of the highest scientific quality in the field of cutaneous pathology. It aims at promoting and maintaining communication between clinicians and research workers. Cutaneous pathology will be interpreted to mean diseases of the epidermis, dermis and cutaneous appendages.
Original scientific articles on diagnostic and experimental cutaneous pathology are invited. Clinico-pathological studies will also be considered for publication.

As a special offer to new subscribers to JOURNAL OF CUTANEOUS PATHOLOGY we can offer you volumes 1 and 2 (1974 & 1975) FREE as long as the stock lasts so you can have the journal right from the beginning.

MUNKSGAARD · COPENHAGEN

Order Form to be sent in an envelope to:

MUNKSGAARD
35, Nørre Søgade
DK-1370 Copenhagen K.
Denmark

JOURNAL OF CUTANEOUS PATHOLOGY

☐ Please enter my subscription to start with volume 3, 1976. Subscription price D.Kr. 320.00 plus postage D.Kr. 14.00 (US$ 56.50 incl. postage).

☐ Please send me volumes 1 and 2 FREE (special offer to new subscribers only).

☐ I enclose ____________ in payment.

Name: ____________

Address: ____________

TEXTBOOK OF DERMATOLOGY

Available again as a reprint of the Second Edition

Edited by Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. *Second Edition*, 1972 (*Reprinted* 1975). 2236 pages, 1100 illustrations. Two volumes. £48.00

'The appearance of the second edition of this book comparatively soon after the first testifies the success with which it has fulfilled a need.... these two volumes are essential to the dermatologist; the trained dermatologist will need them for frequent reference, while for the trainee they should be the sheet-anchor of systematic study.' *British Journal of Dermatology*

'In their second edition the authors have expanded on the knowledge previously presented and have added several quite useful and appropriate sections. The textbook will continue to be a superb reference book which should be available to all dermatologists; it is admirable as a comprehensive reference, detailed, and well-written survey of the field of dermatology.' *The Journal of Investigative Dermatology*

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Aldenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, 81, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: Beare, J.M. & Wilson Jones, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 95, Number 2, August 1976

CONTENTS

Clinical and Laboratory Investigations

PAGE

Kinetics of *In Vitro* Bovine Keratohyalin Synthesis 115
PETER M.ELIAS, WALTER G.KLINGLER AND PAUL M.MONTAGUE

A Study of the Permeability Barrier in Epidermis and Oral Epithelium using Horseradish Peroxide as a Tracer *In Vitro* 123
C.A.SQUIER AND ROSAMUND M.HOPPS

Study of an Inhibiting Factor of Epidermal Profileration in Plucked Skin and Various Tumours of Mice 131
M.GUIGON, E.FRINDEL, J.HEWITT AND M.TUBIANA

Sociological Implications of as Epidemiological Study of Eczema in the City of Birmingham 137
N.C.BOWKER, K.W.CROSS, E.A.FAIRBURN AND M.WALL

Complement Componant Profiles in Urticaria, Dermatitis Herpetifotmis, and Alopecia Areata 145
L.BERRENS, E.JANKOWSKI AND I.JANKOWSKI-BERNTSEN

Pilo-sebaceous Duct Physiology. III. Observations on the Number and Size of Pilo-sebaceous Ducts in Acne Vulgaris 153
W.J.CUNLIFFE, W.D.H.PERERA, P.THACKRAY, M.WILLIAMS, R.A.FORSTER AND S.M.WILLIAMS

Erythropoietic Protoporphyria. Hepatic Implications 157
D.M.MACDONALD AND D.C.NICHOLSON

HL-A Antigens in Patients with Guttate Psoriasis 163
R.C.WILLIAMS, A.W.MCKENZIE, J.H.ROGER AND V.C.JOYSEY

HL-A Antigens in Lichen Planus 169
N.J.LOWE, A.G.CUDWORTH AND J.C.WOODROW

HL-A Antigens and Vitiligo 173
G.RETORNAZ, H..BETUEL, J.P.ORTONNE AND J.THIVOLET

Pharmacology and Treatment

Clinical and Bacteriological Evaluation of a Twice Daily Regimen of Epicillin Capsules for the Treatment of Skin Infections 177
M.EL-ZAWAHRY, ABBAS ABDEL AZIZ, MOHSEN SOLIMAN, S.SHOEIB AND S.SHOEIB AND S.S.TADROS

Erythemia Multiforme in Children. Response to Treatment with Systemic Corticosteroids 181
JAMES E.RASMUSSEN

Studies on the Effect of Salicylic Acid on Normal Skin 187
M.DAVIES AND R.MARKS

Dithranol (Anthralin) Versus Triacetoxyanthracene. Investigations *In vitro* 193
WOLFGANG P.RAAB

Case Reports

Mudi-chood on the Pinnae 197
P.SUGATHAN

Multiple Pigmented Naevi in an Albino 199
M.HALL, V.EUSEBI AND I.D'ANGELO

Leg Ulcers in a Family with both Beta Thalassaemia and Glucose 6-phosphate Dehydrogenase Deficiency 203
S.GANOR AND T.COHEN

Glaucoma Following the Application of Corticosteriod to the Skin of the Eyelids 207
R.BEVIS CUBEY

Society Proceedings

Dowling Club Proceedings 209
(Chairman: DR R.P.WARIN)

Book Reviews 215

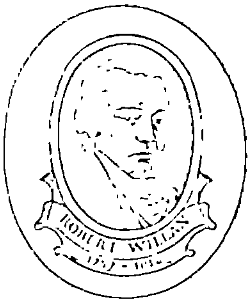
Review

Treatment of Obesity. Appetite Suppressant Drugs and Intestinal By-pass Surgery 219
S.S.BLEEHEN, I.R.EDWARDS AND R.G.CLARK

News and Notices 223

Hex.
11 12.76

Volume 95, Number 3, September 1976



British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

British Journal of Dermatology

EDITOR

DR R. H. CHAMPION
Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

DR J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London
DR RONALD MARKS, Cardiff
DR P.D.MIER, Nijmegen
DR J.N.S.MITCHELL, Royal Air Force
DR W.C.NOBLE, London
DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS

DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 U.K. (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed in Great Britain at the Alden Press, Oxford

IMPORTANT NEW PRODUCT FOR PSORIASIS

olan

Dithranol

Much easier to apply, far easier to remove, and actually more effective.

DITHROLAN is quite simply the most convenient and effective form of dithranol in the treatment of psoriasis.

A careful and painstaking study carried out over the last 5 years at the Beaumont Hospital, Lancaster, demonstrated that a newly developed formulation of dithranol, now introduced as DITHROLAN, was not only very much easier to use than traditional Dithranol Paste, but was actually more effective[1, 2].

DITHROLAN adheres easily to the skin and can be applied quickly and conveniently. Previous applications of DITHROLAN can be removed simply by bathing – obviating the need for oil. As a result, patients actually carry out their treatment.

Dermatologists will appreciate DITHROLAN because of the considerable saving in treatment effort and time, and because of the excellent results obtained.

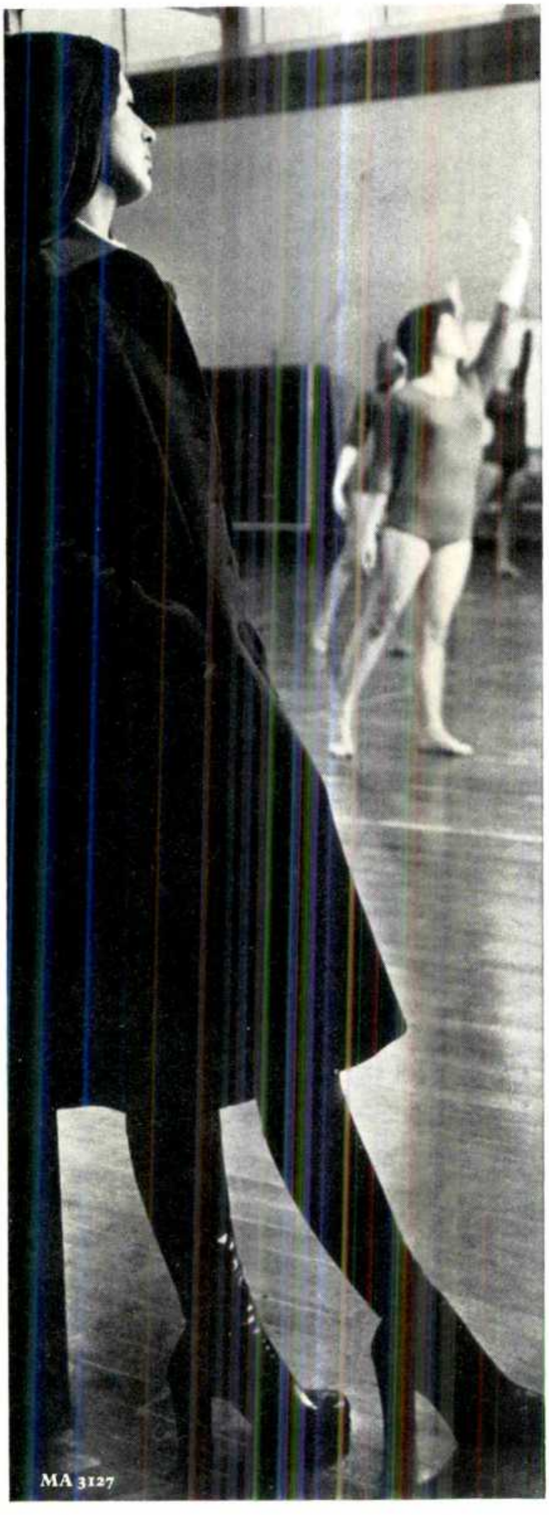
Presentation: DITHROLAN – containing 0·5% Dithranol B.P, 0·5% Salicylic Acid B.P. 90 gram amber glass jar. PL 0173/0024

References: 1. Brit. J. Derm. **93,** *205. 1975.*
2. Dermatology Digest **Feb,** *20, 1976.*

Further information and literature available from:

Dermal Laboratories Limited,
Tatmore Place, Gosmore, Hitchin,
Herts, SG4 7QR, England

Dithrolan



"Pruritus ani and vulvae is a symptom that can be so annoying, embarrassing, and intractable that sufferers are willing to undertake any treatment to get relief."[1]

'Vallergan'

has so many benefits

antipruritic

'Vallergan' relieves the itching and so stops the scratching. 'Vallergan' reduces the need for any form of topical medication including corticosteroids. 'Vallergan' helps prevent exacerbation and secondary infection.

*** analgesic**

'Vallergan' relieves irritation and pain.

*** antihistaminic**

'Vallergan' is most effective in urticaria and other allergic skin disorders.

*** sedative**

'Vallergan' promotes sleep at night.

*** well tolerated**

'Vallergan' is well tolerated by infants, children and adults, including the elderly.

*** presentations**

You can prescribe 'Vallergan' either as tablets or as syrup, available in two strengths.

'Vallergan' for itching, scratching, and irritation in all conditions at all sites, at all ages, whatever the cause

Reference 1 Olansky, S.; in *Current Therapy*; edited by H. F. Conn, London: Saunders, 1969, p.646.

Full information is available on request
'Vallergan' is a trade mark of
May & Baker Ltd Dagenham Essex RM10 7XS
for its preparations of trimeprazine tartrate.

M&B May&Baker

A member of the Rhône-Poulenc Group of Companies

'OUTLOOK IN PSORIASIS'

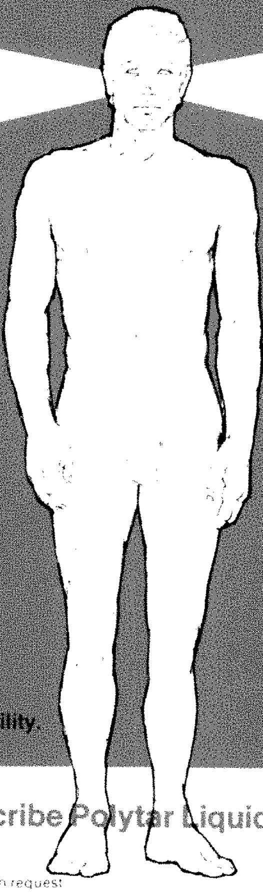
'The time-honoured applications of tar or dithranol and exposure to ultra-violet light remain the basis for local treatment'

Editorial, Brit. med. J. 1974,4,365

Polytar Liquid Psoriasis of the scalp

In a recent teaching hospital study, Polytar Liquid was compared with a standard cetrimide formulation in 50 patients with psoriasis of the scalp. Polytar Liquid proved superior in the removal of scale and the relief of oiliness. Patient acceptability was excellent.

Polytar Liquid combines effective control with patient acceptability



Polytar Emollient Psoriasis of the body

Polytar Emollient is a tar bath additive which does not stain the bath surface, hair, skin or nails. In a double blind controlled trial with 90 patients Polytar Emollient proved superior to liquor picis carb. in terms of patient and nursing staff acceptability.

"Polytar Emollient has a useful part to play in the treatment of psoriasis, particularly in out-patient treatment".

Clin. Trials J. 1970, 4, 438

Polytar Emollient superior to liquor picis carbonis

For psoriasis – prescribe Polytar Liquid and Polytar Emollient

Full information on request

Stiefel Laboratories (UK) Limited 825 Yeovil Road, Slough, SL1 4JA

tarcortin

Cream

succeeds in eczema where others fail

Tarcortin Cream, a non-staining combination of a highly refined coal tar extract and hydrocortisone, produces excellent results in eczema and dermatitis – even where there has been no response to either agent alone. It has been found highly effective in the treatment of delicate skins.

Tarcortin Cream is available in 30g and 45g tubes.

Further information, including Data Sheet, available on request from:

Stafford-Miller

The Professional Relations
Division, Stafford-Miller
Limited, Hatfield, Herts.

In difficult eczema

Dermovate

it's better sooner

Dermovate often succeeds where other topical steroid preparations fail. These pictures, for example, show the response of a long-standing case of vesicular eczema after only five days.

Dermovate contains clobetasol propionate, a steroid shown to be superior to others* in its ability to bring the most stubborn cases swiftly under control.

So treatment need only be of short duration.

And when infection is present or suspected, Dermovate combined with neomycin and nystatin is available as Dermovate-NN.

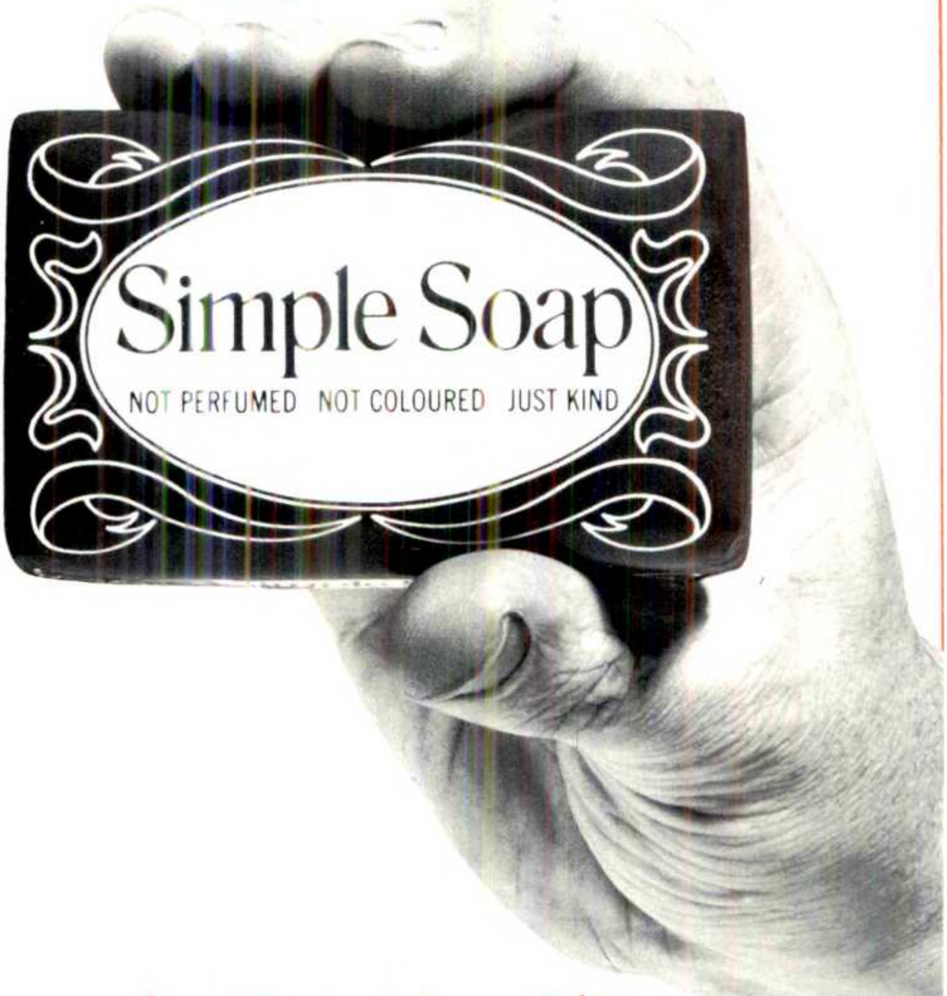
British Journal of Dermatology* (1974) **90, 197

Dermovate is available as Cream and Ointment in 25 and 100 gram tubes
Full prescribing information on Dermovate (trade mark) is available from: Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE

Glaxo

When there is potential danger of skin conditions recurring or being exacerbated through irritation or allergic reaction ......

Simply recommend



for Sensitive Skins

Skin problems may often be exacerbated by the colourants, perfumes or high-lather detergents contained in many brands of 'cosmetic' soap. Simple Soap contains none of these potentially harmful additives—it simply cleanses with the gentle lathering action of pure soap. That's why Simple Soap is widely used by hospitals throughout the country.

Simple Soap is available to your patients at chemists everywhere,
for about 14p and 24p.

A regular supply of professional samples may be obtained on application to:
The Medical Division, Simple Soap Ltd., Hampton, Middlesex.

"The Brocades Representatives cannot be held esponsible for failing to communicate to you the effectiveness and clinical versatility of Pimafucin 2% cream as a opical treatment for fungal skin infections due to yeasts or dermatophytes.

Our hands were tied by the Company which decided hat we must spend more time telling you about our other excellent products Locoid, De-Nol and Cyclospasmol 400.

Yet, as we have known all along, there is a wealth of clinical evidence to support the efficacy of Pimafucin 2% cream. International investigations, embracing a wide variety of fungal skin infections, have consistently demonstrated impressively high cure rates.[1] In a recent British study[2] a 98% cure rate was achieved in 43 patients with culturally proven genital candidosis.

The fact that some doctors are already aware of the value of Pimafucin 2% cream in dermatology – if the recent ncrease in prescriptions is anything to go by – is due more o their perspicacity than ours.

So we apologise for doing you a disservice by not elling you about Pimafucin 2% cream and now want to nake amends, so please give us an opportunity to tell you he full story when we next visit you."

Pimafucin is the brand name for products containing natamycin and is available as 2% cream, vaginal tablets (for candidal, trichomonal and mixed vaginitis), 1% suspension (for oral candidosis) and 2½% suspensio (for pulmonary fungal infections).

[1] Raab, W.P. "Natamycin (Pimaricin), Its Properties and Possibilities in Medicine (1972)."

[2] Masterton G. et al. Brit. Journal of Venereal Diseases (1975) Vol. 51 210.

the cream of the broad spectrum antifungal creams.

For further information, please contact:
Brocades/Great Britain/Limited, Brocades House,
West Byfleet, Surrey. Telephone: Byfleet 45536.

84%

of plantar warts wiped out with Salactol.

Salactol is the most reliable, best documented form of treatment yet developed for warts.

The evidence for Salactol is impressive. In a double blind controlled trial [1] 84% of cases with plantar warts were cured within a 12 week period. Its value is further confirmed in papers [2, 3] *where Salactol is actually recommended by name.*

And it's a British product through and through.

Reliability, convenience and efficacy make Salactol the most widely accepted preparation for the treatment of warts in the UK.

Full information on Salactol is available from:

Dermal Laboratories Limited
Tatmore Place, Gosmore, Hitchin
Hertfordshire SG4 7QR

Salactol contains salicylic acid and lactic acid in a flexible collodion base and is presented in a 10ml bottle (more than enough for a full course of treatment) with a special applicator.

References:

1. Bunney, Mary H. (1971), Practitioner, **207**, 197.
2. Pegum, J. S. (1972), Practitioner **209**, 453.
3. Bunney, Mary H. (1974), Prescribers Journal, **14**, 118.

PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
the modern basis of treatment

'. . . to restrict topical steroid therapy to short periods for getting the disease under control and then to change to tar preparations which may prevent rebound exacerbations.'
Practitioner, 1970, **204**, 625.

New Alphosyl HC cream
for short-term control
The unique descaling, healing and anti-inflammatory properties of Alphosyl, plus the added anti-inflammatory action of hydrocortisone to get the disease swiftly under control.

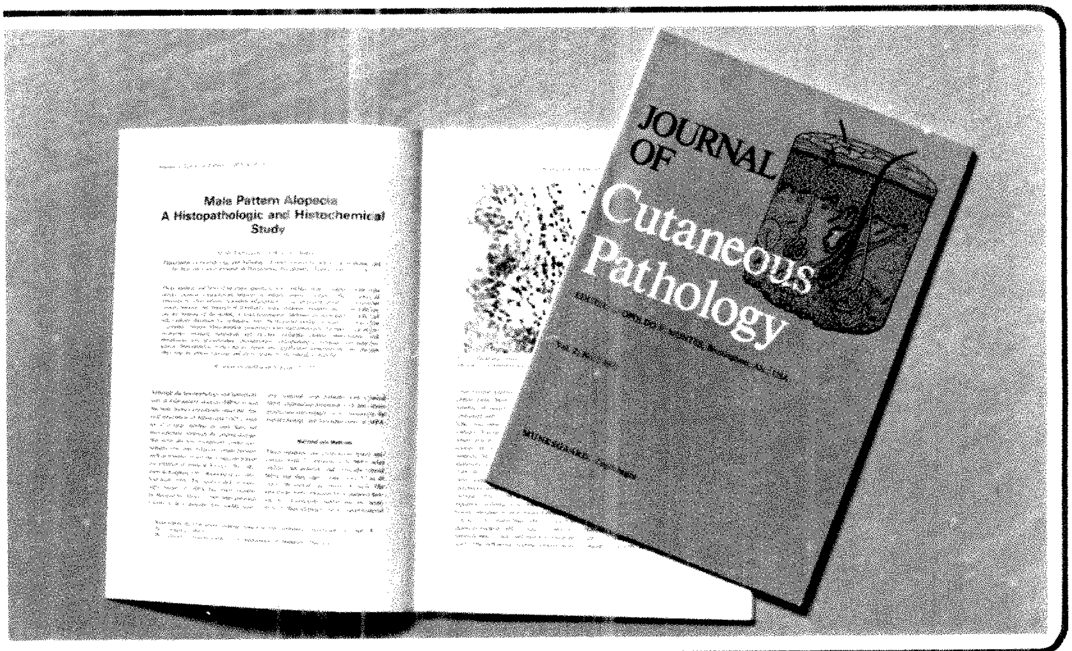
Alphosyl cream
lotion and shampoo for long-term maintenance
'. . . it (Alphosyl) does not have the disadvantage of staining, burning and discolouration that occur with other topical preparations.' Br.med.J., 1967, **4**, 535.

Alphosyl
a whole range of treatment for psoriasis

Alphosyl cream, lotion and shampoo contain a highly refined extract of coal tar and allantoin. Alphosyl HC in addition contains 0.5% hydrocortisone. Please send to us for samples, clinical information and Data Sheets.

Stafford-Miller The Professional Relations Division, Stafford-Miller Limited, Hatfield, Herts.

Dermatology



Journal of Cutaneous Pathology

Editor: Leopoldo F. Montes, Birmingham, Alabama

Journal of Cutaneous Pathology publishes manuscripts of the highest scientific quality in the field of cutaneous pathology. It aims at promoting and maintaining communication between clinicians and research workers. Cutaneous pathology will be interpreted to mean diseases of the epidermis, dermis and cutaneous appendages.

Original scientific articles on diagnostic and experimental cutaneous pathology are invited. Clinico-pathological studies will also be considered for publication.

As a special offer to new subscribers to JOURNAL OF CUTANEOUS PATHOLOGY we can offer you volumes 1 and 2 (1974 & 1975) FREE as long as the stock lasts so you can have the journal right from the beginning.

MUNKSGAARD · COPENHAGEN

Order form to be sent in an envelope to:
MUNKSGAARD
35, Nørre Søgade
DK-1370 Copenhagen K.
Denmark

JOURNAL OF CUTANEOUS PATHOLOGY

☐ Please enter my subscription to start with volume [illegible], 19[illegible]. Subscription price D.Kr. [illegible] plus postage D.Kr. 14.00 (US$ [illegible] incl. postage).

☐ Please send me volumes 1 and 2 FREE (special offer to new subscribers only).

☐ I enclose ______ in payment.

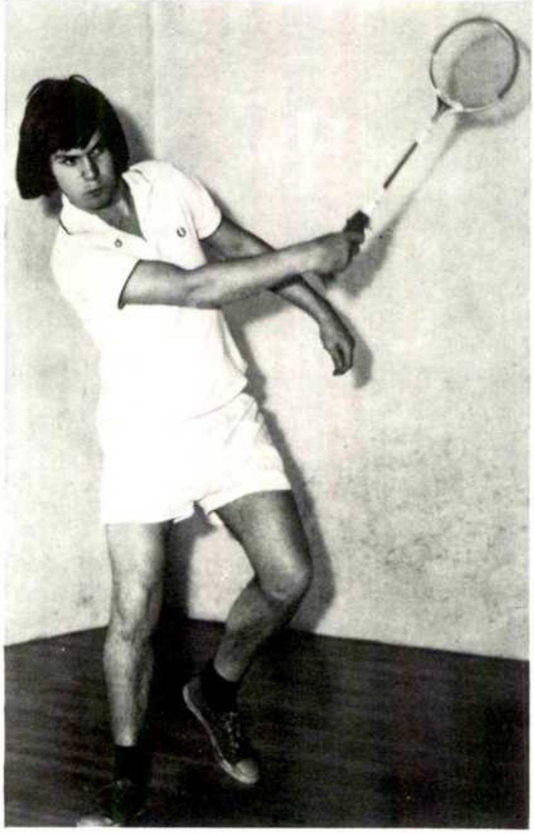
Name: ______

Address: ______

______

______

ACNE?



prescribe patient confidence

"Acne is an almost universal complaint in adolescence."[1]

"Many patients when they seek the help of their doctor are depressed, discouraged and disillusioned. Their own efforts at treatment have failed, or the first prescription was not the cure they anticipated. In the management of acne the patient must appreciate that he or she may have to persevere with treatment throughout their teens, and it should be emphasised that in this way one hopes to limit as far as possible the severity of the lesions and prevent severe or permanent scarring."[2]

When patients like these ask for your help remember that clinical reports show that acne responds to Quinoderm.

Available as Quinoderm
with **Hydrocortisone Cream 30G.** for initial treatment.
Quinoderm Cream 25G. for subsequent treatment.

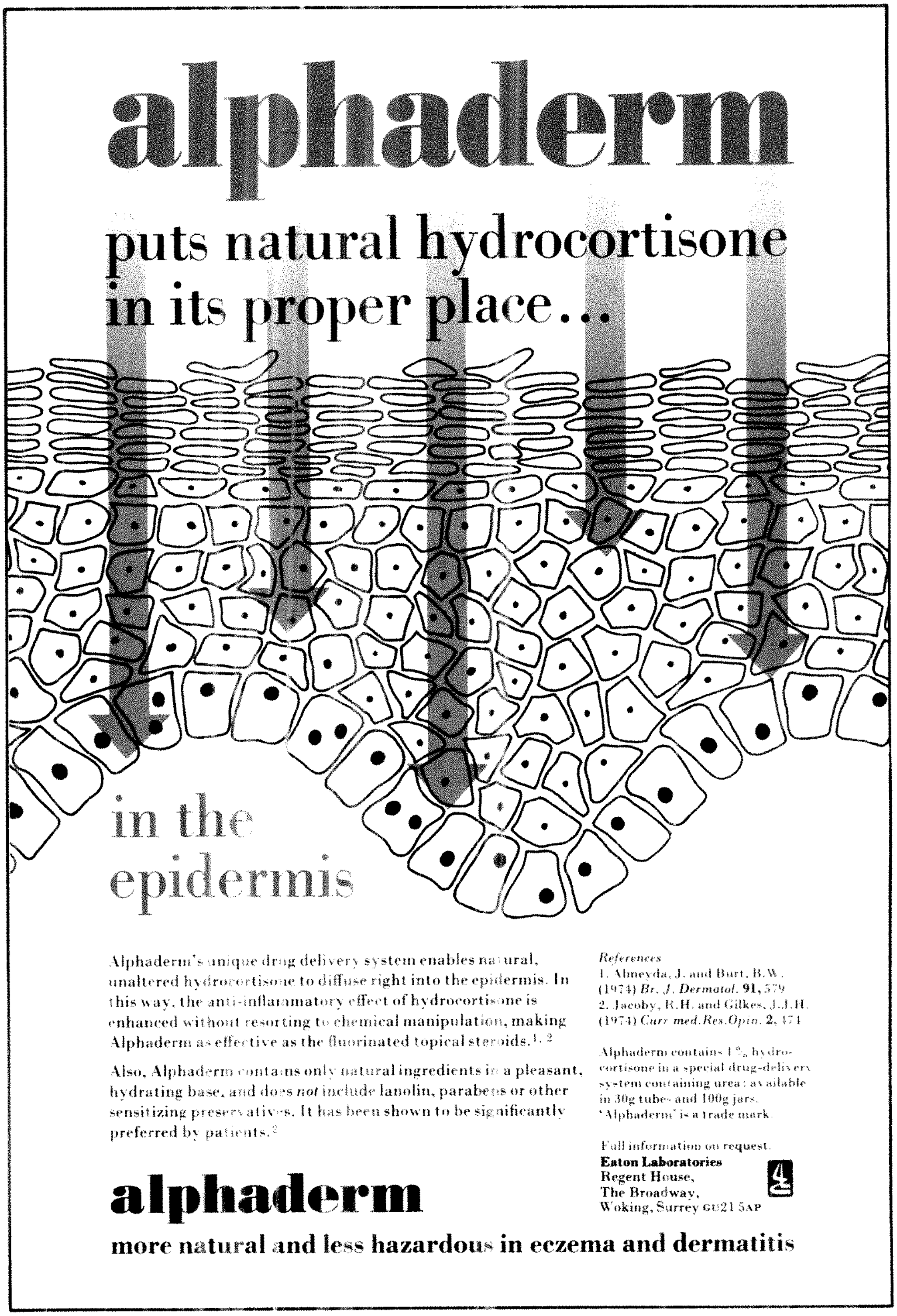
1, 2. Practitioner 1970, 204, 639

Quinoderm Hydrocortisone 30G.
Benzoyl Peroxide 10.0%
Potassium Hydroxyquinoline, Sulphate 0.5%
Hydrocortisone 1%
in an astringent cream base.

Quinoderm Cream 25G.
Benzoyl Peroxide 10.0%
Potassium Hydroxyquinoline Sulphate 0.5%
in an astringent cream base.

Quinoderm

For further information and literature please write to THE QUINODERM COMPANY, OLDHAM, LANCS.



alphaderm

puts natural hydrocortisone in its proper place...

in the epidermis

Alphaderm's unique drug delivery system enables natural, unaltered hydrocortisone to diffuse right into the epidermis. In this way, the anti-inflammatory effect of hydrocortisone is enhanced without resorting to chemical manipulation, making Alphaderm as effective as the fluorinated topical steroids.[1, 2]

Also, Alphaderm contains only natural ingredients in a pleasant, hydrating base, and does *not* include lanolin, parabens or other sensitizing preservatives. It has been shown to be significantly preferred by patients.[2]

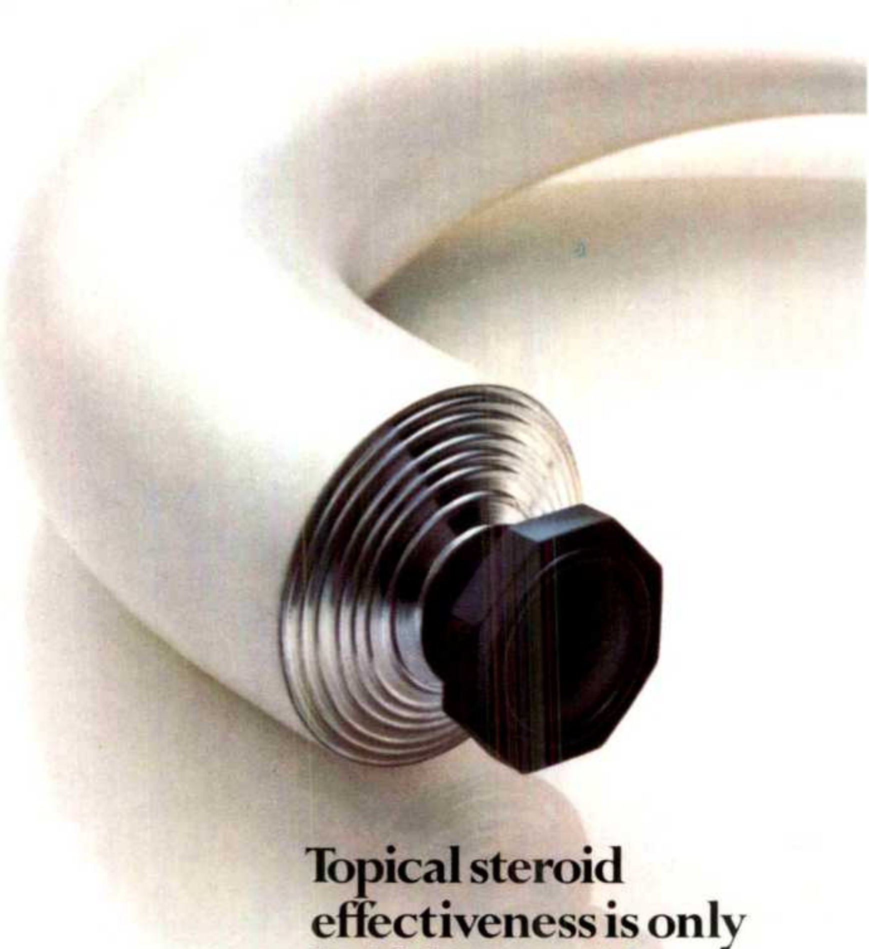
References
1. Almeyda, J. and Burt, B.W. (1974) *Br. J. Dermatol.* **91**, 579
2. Jacoby, R.H. and Gilkes, J.J.H. (1974) *Curr med.Res.Opin.* **2**, 474

Alphaderm contains 1% hydrocortisone in a special drug-delivery system containing urea: available in 30g tubes and 100g jars.
'Alphaderm' is a trade mark.

Full information on request.
Eaton Laboratories
Regent House,
The Broadway,
Woking, Surrey GU21 5AP

alphaderm

more natural and less hazardous in eczema and dermatitis



Topical steroid effectiveness is only half the Locoid story

...the other half is the relative freedom from topical side effects which are often encountered with the fluorinated steroids. Locoid offers the same level of success as these potent preparations – without fluorination.

Locoid is a trade mark for hydrocortisone 17-butyrate.
Full information available on request:
Brocades (Great Britain) Ltd.,
Brocades House, West Byfleet, Surrey, KT14 6RA.
Telephone: Byfleet 45536.

locoid

the non-fluorinated way to topical steroid success

Canesten®
clotrimazole
broad-spectrum antifungal

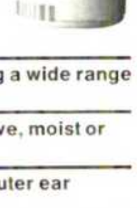
More and more doctors are finding Canesten a highly effective and trouble-free treatment for all the fungal infections commonly seen in hospital practice.

In Gynaecology:
6-day Canesten
the only effective 6-day treatment for candida and mixed vaginal infections.

In Dermatology:
broad-spectrum Canesten
now the most widely prescribed treatment for fungal skin infections.

The new solution for fungal skin infections

New Canesten Solution



As effective as Canesten cream in treating a wide range of fungal skin infections

Especially suitable for applying to sensitive, moist or hairy areas

Also suitable for fungal infections of the outer ear (otitis externa)

Both the cream and the new solution are easy to apply, non-greasy, non-staining and odourless

Bayer

Further information, including data sheet, is available from
Bayer UK Limited, Pharmaceutical Division,
Haywards Heath, West Sussex RH16 1TP
Telephone: Haywards Heath (0444) 57911
® Registered Trade Mark of Bayer Germany

British Journal of Dermatology (1976) **95**, 225.

Clinical and Laboratory Investigations

Epithelioma adenoides cysticum: genetic update

DAVID E.ANDERSON AND JAMES B.HOWELL

The University of Texas System Cancer Center, M.D.Anderson Hospital and Tumor Institute,
Houston, Texas and Southwestern School of Medicine, The University of Texas Health Science Center,
Dallas, Texas, U.S.A.

Accepted for publication 2 January 1976

SUMMARY

Epithelioma adenoides cysticum (EAC) is a well-known genodermatosis which follows an autosomal dominant mode of inheritance. Questions still remain, however, concerning an apparent excess of females with EAC. An analysis of nineteen previously published pedigrees and one newly ascertained pedigree, which in the aggregate included over 175 cases of EAC, indicated no excess of affected females, but rather, a marked deficit of affected males. This deficiency was not the consequence of sex linkage or decreased viability, and was most evident in large pedigrees and sibships, sibships not including the probands, and late birth orders. In these situations, the penetrance of EAC in male gene carriers was only 50%, but was close to 100% in female gene carriers. Under maximum detection conditions, i.e. small pedigrees, small sibships, sibships containing the proband, and early birth orders, the penetrance in males increased to 85% and was again close to 100% in females. These findings suggested that the deficit of affected males was best explained by a combination of lessened expressivity and penetrance, effects which were magnified under situations of poor detection. A review of familial cases of EAC indicated no consistent associated anomalies such as those that characterize the naevoid basal cell carcinoma syndrome.

Epithelioma adenoides cysticum is a rare genodermatosis, characterized by multiple tumours that develop chiefly about the nasolabial folds, face and eyelids, and also on the scalp and trunk. The tumours, usually first noted during puberty, may range in size from 2 to 30 mm, the latter size probably representing a coalescence of smaller lesions. The tumours and the condition have been called by a variety of terms including epithelioma adenoides cysticum, multiple benign cystic epithelioma, multiple trichoepithelioma, Brooke's tumour, and Brooke–Fordyce trichoepithelioma.

Turban or Ancell-Spiegler cylindromas and trichoepitheliomas, although being clinically and microscopically distinguishable and presumably arising from different structures, have long been considered to be different tumour mainifestations of the same condition (Cockayne, 1933; Savatard, 1938). This association is evident by the occurrence of both tumour types in the same patient and by the occurrence of trichoepitheliomas in one member and cylindromas alone or in combination with

---

Reprint requests to Dr David E.Anderson, Section of Medical Genetics, Department of Biology, The University of Texas System Cancer Center, M.D.Anderson Hospital & Tumor Institute, Houston, Texas 77030, U.S.A.

trichoepitheliomas in another member of the same family. Welch, Wells & Kerr (1968), and Rasmussen (1975) presented strong familial data supporting the concept that both tumours were indeed different phenotypic manifestations of the same genetic defect. Furthermore, that the tumours may be histologically variable is not unusual since Gartler *et al.* (1966) provided evidence of their multicellular origin, and as stated by Welch *et al.* (1968), the histological characteristics of a tumour will depend on the origin of the cell from which the genetically determined lesion develops. For present purposes, the condition characterized by trichoepitheliomas and/or cylindromas will be referred to as epithelioma adenoides cysticum (EAC).

EAC has a widespread distribution, having been reported in most of the European peoples and in their North American descendants. It has seldom been reported in Orientals and Negroes (Cockayne, 1933). The disorder is accepted as following a dominant mode of inheritance, but questions still remain concerning the apparent excess of affected females. Cockayne (1933) tentatively attributed the excess to a sex-linked recessive lethal gene. Serra, Bernardi-Ronzoni & Paoletti (1957) and Wolken, Spivey & Blodi (1969) attributed it to penetrance differences between the sexes. However, Goldman (1940), Fliegelman & Kruse (1948), Gaul (1953), and Welch *et al.* (1968), reported no obvious excess of affected females or sex limitation in five large pedigrees. Whether the excess in some but not all families has biological significance or is the consequence of bias or sampling variation has not been determined.

Past reports of familial-occurring EAC have usually referred only to a single pedigree, or rarely, to two pedigrees. A series of pedigrees could provide evidence of differences or trends which would not be readily detected in a single pedigree, and might, therefore, indicate if the sexes were indeed differentially affected.

MATERIALS

The primary data were derived from previously published pedigrees which were required to include information on the disease status of the parents and sex of the affected parent, sibs, and/or children of a patient. Many of the early reports on familial occurrences were not usable because they concentrated on affected individuals and failed to provide information on other family members. Families initially reported as examples of EAC but which are now accepted as representing the naevoid basal cell carcinoma syndrome were also obviously not used. Seventeen reports were found containing nineteen pedigrees that met the above criteria. Not all information included in the pedigrees was utilized. The latest generations, where the children at risk were generally below 10 years of age, were excluded from the tabulations (Goldman, 1940, Generation IV; Gaul, 1953, Generation V; Serra *et al.*, 1957, Generation V; Welch *et al.*, 1968, Generation V in Pedigree A and Generation IV in Pedigree B; Wolken *et al.*, 1969, Generation VI: Rasmussen, 1975, Generation IV). These young generations were excluded since the age range was much too young to provide reliable estimates of segregation ratios.

One additional, previously unpublished, pedigree was ascertained from a review of the medical records at M.D.Anderson Hospital. The proband was a 28-year-old man with multiple, small, flesh-coloured tumours of the entire scalp, nasolabial folds, eyelids, ear, back, axilla, and a large one on the left anterior chest. The tumours had been present since the age of 17. Except for having angio-oedema, findings from his history and physical examination were otherwise normal. A radiographic survey of the skull, mandibles, spine, and chest revealed no abnormalities. Multiple excision biopsies of tumours of the eyelid, ear, nose, scalp and axilla were microscopically interpreted as representing epithelioma adenoides cysticum. His 32-year-old brother was also examined. The brother had similar tumours but they were much fewer in number. They were confined to the right pre-auricular and neck areas; his history and a physical examination including a radiographic survey disclosed no abnormal findings. The family history was significant in that similar tumours were reported in their mother, maternal grandmother, three of four maternal aunts, and in two of four maternal uncles.

RESULTS

The twenty available pedigrees were first tested for homogeneity with regard to the secondary sex ratio and male and female segregation ratios. No evidence of heterogeneity was evident except in the case of the male segregation ratios ($P<0.05$) which was explicable on the basis of pedigree size and will be discussed later. Since the pedigrees agreed with the null hypotheses being tested and with one another, it was concluded that they all referred to a dominantly inherited mutation. But in the absence of a specific histological, clinical, biochemical, or genetic marker, it could not be determined for certain whether all pedigrees referred to the same mutation.

EAC in males and females

The pedigrees included a total of 88 sibships, each from an affected parent. These sibships were classified according to whether they involved an affected father or affected mother. Affected fathers were much less frequent than affected mothers (33 vs 55), frequencies which differed importantly from the secondary sex ratio, where 45 or 51·4% of the parents were expected to be male ($P<0.01$). The fathers with EAC produced a total of 66 sons, 33% of whom manifested EAC. These frequencies were similar to those resulting from affected mothers, who had a total of 148 sons, 34% of whom manifested EAC. The affected fathers also produced a total of 69 daughters, 52% of whom had EAC, similar to the results from affected mothers where EAC occurred among 49% of 140 daughters. In view of the close similarity of these results, the two groups of parents were combined as shown in Table 1. Male and female children were not combined because the disease frequency of 34% among all males was significantly less than the 50% among all females ($P<0.01$). Moreover, the ratio of affected to unaffected male children deviated significantly from the expected 1:1 ratio if EAC were assumed to be transmitted as an autosomal dominant trait ($P<0.01$); the ratio for female children was totally compatible with this expectation ($P>0.95$).

The sex difference in the occurrence of EAC was apparently not the consequence of an abnormal sex ratio. The sibships contained a total of 214 males and 209 females, which compared closely with expected frequencies of 217 males and 206 females ($P>0.70$). No evidence of sex linkage was discernible because affected fathers readily produced affected sons. The absence of either sex linkage or evidence of decreased viability in males tended to rule against the sex-linked recessive lethal gene suggested by Cockayne (1933). EAC is apparently transmitted as an autosomal dominant trait, but not as a regular dominant, because EAC is much less frequent in males than females (Table 1).

Table 1 further indicated that when males belonged to the proband's sibship, the disease frequency (43%) agreed with expectation ($P>0.30$), but when they belonged to other sibships, the frequency of EAC declined to 32%, well below expectation ($P<0.01$). Among females, EAC was slightly elevated (57%) in the proband's sibship, but still within the realm of chance variation ($P>0.30$), while the rate in other sibships (47%) was close to the expected rate ($P>0.50$). The male:female sex ratio of 47:53 in the proband's sibship was slightly distorted in favour of females, whereas the ratio of 167:156 in other sibships was normal.

A classification was next made according to the birth order of affected and unaffected male and female children (Table 2). For males, the disease occurrence through birth ranks 1 to 3 was in agreement with expectation ($P>0.10$), but at rank 4 a noticeable decrease was observed. The occurrence

TABLE 1. Frequency of epithelioma adenoides cysticum in male and female children from an affected parent* and whether they belonged to the proband's sibship or to some other sibship

	Male		Female	
Reference	Affected	Unaffected	Affected	Unaffected
Fordyce, 1892	0	4	0	0
Miller, 1915	1	0	2	0
Savatard, 1922	0	3	3	3
Savatard, 1938	5	1	5	2
Goldman, 1940	3	2	4	3
Forman, 1942	1	2	2	4
Fliegelman & Kruse, 1948	3	4	5	6
Gaul, 1953	12	11	16	10
Joseph, 1956	2	1	0	1
Serra *et al.*, 1957	22	53	26	28
Schnyder, 1966	2	2	2	2
Ziprkowski & Millet, 1967	1	0	2	2
Welch *et al.*, 1968	12	33	19	24
Wolken *et al.*, 1969	2	16	10	8
Epinette & Bennett, 1972	2	3	1	5
Gottschalk *et al.*, 1974	0	0	1	2
Rasmussen, 1975	2	0	0	2
Present report	3	6	6	3
Proband's sibship	20	27	30	23
Other sibship	53	114	74	82
Totals	73	141	104	105

* Individuals who were phenotypically unaffected but had an affected parent and affected children were counted as unaffected in their sibship tabulation, but were considered to be a transmitting parent. Probands were excluded from their respective sibships but their children were counted.

of EAC in females, although slightly increased in the first two birth ranks, was compatible with expected frequencies, whether birth order was early or late. Stating it differently, the penetrance of EAC in males through birth ranks 1 to 3 averaged 85%, but after these ranks it averaged only 50%. The penetrance for females was high at all birth ranks.

Similar results were observed when a classification was made for sibship or pedigree size. In small sibships of size 1–2 or in pedigrees where the expected number of affected children was less than 5, males had close to normal affection rates and high penetrance values, but for larger sibships or pedigrees the disease frequency was again markedly diminished and was distinctly less than expected. Females manifested slightly increased disease frequencies in small sibships and pedigrees, and frequencies in agreement with expectation in larger sibships or pedigrees.

An attempt was also made to determine if the two sexes differed in their age at onset of EAC. Information on this age was available on fewer males (25) than females (38), but the sexes exhibited similar ages. In males, the age at onset ranged from 4 to 27 years and averaged $13{\cdot}4 \pm 1{\cdot}3$ years, while in females the age at onset ranged from 6 to 26 years and averaged $14{\cdot}5 \pm 0{\cdot}8$ ($P > 0{\cdot}50$).

A difference in clinical expression of the disease was another possibility to explain the sex difference in EAC, but this proved impractical to assess quantitatively. The descriptions were not adequate and

TABLE 2. Observed distribution of epithelioma adenoides cysticum according to sex and birth order tested against a 1:1 ratio expected with dominant inheritance

Birth order	Male				Female			
	Affected	Unaffected	Total	χ^2	Affected	Unaffected	Total	χ^2
1	19	31	50	2·9	19	15	34	0·5
2	21	18	39	0·2	19	13	32	1·1
3	10	18	28	2·3	12	11	23	0·04
4–5	7	24	31	9·3*	20	24	44	0·4
6–7	5	21	26	9·8*	11	12	23	0·04
8+	6	9	15	0·6	11	13	24	0·2
Total	68	121	189		92	88	180	

* $P<0{\cdot}01$.

the examinations were obviously directed toward females at the expense of males, and in one pedigree (Gaul, 1953), although 25 subjects were examined, their sex was not identified.

Associated anomalies

No anomalies were consistently observed in familial patients with EAC, although admittedly not many of them were intensively investigated for associated features. One thoroughly examined patient was described in Case 1 reported by Fliegelman & Kruse (1948), but no anomalies were observed. One autopsied patient was found to have multiple cysts of the lungs and kidneys (Gray & Helwig, 1963). Physical and radiographic examinations of our patient and his brother revealed no other abnormalities. A number of dermatological findings, in addition to EAC, have been previously described, but the only ones that were observed in more than one patient involved pigmentation anomalies. Gaul (1953) reported spotty pigmentation of the buttocks in one patient and hyperpigmentation of the sides of the neck in another. Fliegelman & Kruse (1948) observed a pigmented area similar to a café-au-lait spot in one patient. A similar area was also described in an unaffected family member who was only 8 years of age at examination. Another young family member had a depigmented area on the thigh. No pigmented or depigmented areas were observed in our two patients. Wolken *et al.* (1969) noted cervical carcinoma in four of twenty-six women in one family, but two of the four did not have EAC. Some other incidental associations involved a patient with EAC and multiple fibromas (Ruggles, 1910) and EAC occurring in association with xeroderma pigmentosum (Withers & Coleman, 1920).

DISCUSSION

From these various but related results, it is apparent that males have only a slightly reduced frequency of EAC and a reasonably high penetrance level when they belong to the proband's sibship, a small sibship or pedigree, or have early birth order, but when sibship or pedigree size is large, birth order is late, or they belong to a sibship not containing the proband, their disease frequency is much reduced. Females, however, show only a slight and insignificant excess of EAC in the categories where the male rates are much reduced. These differences, in view of the absence of reduced viability in males or of sex linkage, suggest that the differences in affection rates between males and females can be best explained by a combination of penetrance and detection differences. That EAC is detectable in about 85% of male gene carriers under maximum detection conditions, i.e. small sibship or pedigree size, early birth order, or when they belong to the proband's sibship, while apparently all female carriers exhibit EAC under the same conditions, suggests that males actually have a reduced expression and penetrance of EAC. If males were generally less apt to seek attention for their skin condition and/or were not available for examination during a family study of EAC because of employment or place of residence, the effect of this lessened expressivity would then be magnified and a deficit of affected males would be recorded. This could explain the results for large sibships or pedigrees, late birth order, or sibships not containing the proband, all of which were characterized by marked deficits of affected males. Lessened expressivity, penetrance, and detection could also explain the deficit of affected males and the consequent apparent excess of affected females among previously reported sporadic cases (Savatard, 1922; Cockayne, 1933), and in the present analyses, the deficit of affected fathers, examined males, and males for whom age information was available.

This lessened expressivity and penetrance in males implies that their disease is perhaps more variable and less extensive on the average than in females. They may develop fewer and/or smaller tumours, the tumours may be located in unobtrusive areas and not be cosmetically objectionable, or multiple tumour development might be later developing in males than in females. In the present data, the age at onset, although having a similar range and average in the two sexes, was 1·6 times more variable in males than in females. Differences in the variability and extent of the disease in males and females are illustrated by the involvement in a woman whose 80-year-old father had only two small scalp tumours and minimal facial involvement (Welch *et al.*, 1968). Savatard (1938) alluded to similar differences between male and female patients. Welch *et al.* (1968) also reported that some patients were not aware of small lesions in the scalp. This evidence of lessened expressivity in males is admittedly meagre, but there must be some reason other than mere reluctance on the part of males to seek medical attention. The most reasonable explanation is that their disease is less severe, less objectionable, and therefore less frequently attended. Why it should be less severe is not known, but endocrine factors could be a factor. The tendency for the tumours to be noted first in puberty and to become progressively more extensive with age is compatible with an endocrine influence expressed differentially in the sexes. Lesions in the mother of Joseph's (1956) patient were said to improve and disappear during the menopause. Gray & Helwig (1963) also noted the apparent disappearance of an occasional tumour in three of their fifty patients, but made no mention of the sex or age of these patients. Some of these questions concerning sex and endocrine differences might be more easily resolved had attention in earlier reports been directed to these possibilities.

Although females exhibited affection rates that were statistically compatible with those expected under dominant inheritance, a slight but consistent excess of affected females was observed under conditions of maximum detection. In the present analysis, male and female probands were excluded from all tabulations, but this may not have been sufficient to overcome a possible over-reporting of affected females. Such over-reporting could have occurred if some families included primary as well as secondary probands and the secondary probands were not identified. For example, a mother under observation and treatment for EAC could seek medical attention for her pubertal daughter in whom tumours were beginning to develop. This parent-offspring combination could then have led to a more extensive family study. The mother would likely have been recorded as the index parent, or proband, when in fact, both should have been so recorded. If such mother-offspring combinations were more frequent than father-offspring combinations, which is conceivable since more affected mothers were recorded than affected fathers and more females underwent examination than males, the resulting family data would then include a slight excess of affected female children, and perhaps a slight distortion in the sex ratio. This type of phenomenon might explain the slight excess of females and affected females in the proband's sibship. No such excesses were observed in other types of sibships, or in large sibships or pedigrees where such over-reporting would not be expected.

Unlike the naevoid basal cell carcinoma syndrome, EAC is not characterized by the occurrence of multiple rodent ulcers (Howell & Anderson, 1976). The two disorders also differ in the occurrence of associated anomalies, which are frequent and characteristic of the naevoid basal cell carcinoma syndrome and are apparently absent in EAC. The two disorders also differ in that the syndrome follows an autosomal dominant mode of inheritance with high penetrance in both sexes, whereas EAC is characterized by an autosomal dominant mode of inheritance with high penetrance in females and reduced penetrance in males, which under maximum detection conditions appears to be about 85%.

ACKNOWLEDGMENTS

The authors acknowledge the technical assistance of Louise C.Overton (deceased). This study was supported in part by Grant GM-19513, C-1 from the National Institutes of Health.

REFERENCES

COCKAYNE, E.A. (1933) *Inherited Abnormalities of the Skin and its Appendages*, pp. 22, 301. Oxford University Press, London.

EPINETTE, W.W. & BENNETT, J.E. (1972) Anaplastic 'pilar' carcinoma arising in trichoepithelioma. *Cutis*, 10, 446.

FLIEGELMAN, M.T. & KRUSE, W.T. (1948) Hereditary multiple benign cystic epithelioma. *Journal of Investigative Dermatology*, 11, 189.

FORDYCE, J.A. (1892) Multiple benign cystic epithelioma. *Journal of Cutaneous Disease*, 10, 459.

FORMAN, L. (1942) Familial and congenital epithelioma in the distribution of epithelioma adenoides cysticum, and showing some of the histological changes of the latter. In: *Proceedings of the Royal Society of Medicine, Part* 1 (Ed. by R.Davies-Colley and M.Davidson), p. 261. Longmans, Green and Co., London.

GARTLER, S.M., ZIPRKOWSKI, L., KRAKOWSKI, A., EZRA, R., SZEINBERG, A. & ADAM, A. (1966) Glucose-6-phosphate dehydrogenase mosaicism as a tracer in the study of hereditary multiple trichoepithelioma. *American Journal of Human Genetics*, 18, 282.

GAUL, E. (1953) Multiple benign cystic epithelioma, 'The Indiana Family'. *Archives of Dermatology and Syphilology*, 68, 517.

GOLDMAN, H.G. (1940) Multiple benign cystic epithelioma. *Journal of the American Medical Association*, 115, 2253.

GOTTSCHALK, H.R., GRAHAM, J.H. & ASTON, E.E. (1974) Dermal eccrine cylindroma, epithelioma adenoides cysticum of Brooke, and eccrine spiradenoma. *Archives of Dermatology*, 110, 473.

GRAY, H.R. & HELWIG, E.B. (1963) Epithelioma adenoides cysticum and solitary trichoepithelioma. *Archives of Dermatology*, 87, 102.

HOWELL, J.B. & ANDERSON, D.E. (1976) Transformation of epithelioma adenoides cysticum into multiple rodent ulcers: Fact or fallacy? A historical vignette. *British Journal of Dermatology*, 94, 233.

JOSEPH, H.L. (1956) Epithelioma adenoides cysticum with basal-cell carcinoma. *Archives of Dermatology*, 74, 113.

MILLER, J.W. (1915) Multiple benign cystic epithelioma, with a report of four cases. *Journal of Cutaneous Disease*, 33, 462.

RASMUSSEN, J.E. (1975) A syndrome of trichoepitheliomas, milia, and cylindromas. *Archives of Dermatology*, 111, 610.

RUGGLES, E.W. (1910) Preliminary report upon a case of multiple benign cystic epithelioma and multiple fibroma in the same patient. *Journal of Cutaneous Disease*, 28, 248.

SAVATARD, L. (1922) Epithelioma adenoides cysticum. *British Journal of Dermatology and Syphilology*, 34, 381.

SAVATARD, L. (1938) Epithelioma adenoides cysticum. *British Journal of Dermatology*, 50, 333.

SCHNYDER, U.W. (1966) Tumoren der Haut in genetischer Sicht. *Praxis*, 55, 1478.

SERRA, A., BERNARDI-RONZONI, M.G. & PAOLETTI, R. (1957) Ricerche genetische sull'epithelioma adenoid cistico benigno. *Acta Geneticae Medicae Gemellologiae (Roma)*, 6, 367.

WELCH, J.P., WELLS, R.S. & KERR, C.B. (1968) Ancell-Spiegler cylindromas (turban tumours) and Brooke-Fordyce trichoepitheliomas: Evidence for a single genetic entity. *Journal of Medical Genetics*, 5, 29.

WITHERS, W.M. & COLEMAN, W.G. (1920) XIII. Multiple benign cystic epithelioma associated with xeroderma pigmentosum. *Archives of Dermatology and Syphilology*, 2, 27.

British Journal of Dermatology (1976) 95, 233.

Transformation of epithelioma adenoides cysticum into multiple rodent ulcers: fact or fallacy

A HISTORICAL VIGNETTE

J.B.HOWELL* AND DAVID E.ANDERSON†

* Southwestern Medical School, The University of Texas Health Science Center, Dallas, Texas and
† The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, U.S.A.

Accepted for publication 2 January 1976

SUMMARY

This historical study has examined the persistent controversy about the propensity of epithelioma adenoides cysticum (EAC) to transform into multiple rodent ulcers, by reviewing cases reported through the years that seemed to support the idea. After focusing on the biological behaviour and natural history of the tumours and comparing them with our present knowledge of the behaviour of EAC, we believe that these cases were incorrectly diagnosed. Rather than EAC, they were probably examples of the naevoid basal cell carcinoma syndrome. The latter syndrome was actually established by the recognition that patients who have multiple rodent ulcers that appear early in life and behave aggressively usually have a constellation of associated developmental defects and that the tumours should be classified as genetically determined basal cell carcinomas and not as EAC.

Interest in epithelioma adenoides cysticum (EAC) was stimulated in 1892 by two nearly simultaneous lectures and publications, one in England by Brooke (1892), and the other in the United States by Fordyce (1892). This fascinating dermatological condition was poorly understood and several cases were reported under a variety of terms (Brooke, 1892). Nevertheless, one feature characterized all these cases, the tumours were benign. In fact, Fordyce (1892), who called the condition multiple benign cystic epithelioma, remarked that 'the entire course of the affection is so free from any evidence of malignancy that the word benign seems an especially happy one to qualify the term epithelioma which custom has associated with malignancy.'

Yet, a controversy about just this benign characteristic soon arose and lasted many years. Authorities generally agreed that EAC tumours were benign, but reports of puzzling cases of EAC periodically appeared in which several of the tumours developed into rodent ulcers (basal cell carcinomas).

Not until 1959, when Howell & Caro established a rare, hereditary syndrome composed of multiple naevoid basal cell carcinomas (NBCC), jaw cysts and skeletal and other developmental defects, were these curious cases adequately explained: these lesions were not EAC but were a unique type of

Reprint requests to: Dr J.B.Howell, 414 Medical Arts Building, Dallas, Texas 75201, U.S.A.

multiple rodent ulcer resulting not from exposure to a usual carcinogen but from a genetic defect. Because the behaviour of the tumours described in the earlier reports paralleled that of the syndrome tumours, those early cases were most likely examples of the NBCC syndrome, as it is now called.

Confusion of NBCC with EAC tumours undoubtedly arose because of overlapping histological characteristics of the two conditions making differentiation difficult. These tumours were and are still easily confused by the unsuspecting diagnostician. Thus, Howell and Caro suggested that the earlier reports be re-evaluated.

In the present historical vignette we re-evaluate several cases whose descriptions we believe are consistent with a retrospective diagnosis of NBCC syndrome. We also outline the chronological development of information accumulated from these unusual cases and also from reports and discussions that at the time seemed unrelated.

The cases of EAC reported before Brooke's (1892) and Fordyce's (1892) reports varied in characteristics such as distribution, colour, and histological features of the lesions as to preclude a definitive description of the condition (Brooke, 1892). As Brooke pointed out, 'it has been the subject of several investigations, but so far without any definite conclusions having been arrived at as to its pathological anatomy and relations, its source and site of origin, the earliest and latest dates of its commencement, its possible modifications, macroscopic and microscopic, and the limits of its distribution.'

The debate about the propensity of EAC tumours to transform into locally malignant rodent ulcers began with a case report by White (1894). The patient, a 45-year-old woman, had approximately 50 lesions mainly on her face but also on her back, upper trunk and arms. At the age of 24 years she had first noticed on her face a few 'pimples,' which slowly grew larger. During the next 18 years these were periodically joined by new tumours that also grew.

According to Brooke, the maximum size of EAC tumours was that of a pea. Several of the tumours in White's patient were about 1 cm in diameter and one was about 2·5 cm in diameter, distinctly larger than would have been expected for EAC. An even more remarkable difference was the recent growth and transformation of three or four older lesions so that they appeared as 'ordinary epithelioma in several of its advanced clinical phases, *viz.* scaling, crusting, and open, deep ulcerative destruction of the whole skin.'

Despite the presence of locally malignant tumours and the general belief that EAC was benign, White diagnosed the condition of his patient as EAC based on a histological study by Bowen. The marked difference between the behaviour of these tumours and that of more typical EAC tumours naturally led White to question whether EAC could any longer be considered benign. And, if tumours of EAC could become malignant, would only some lesions develop into rodent ulcers by chance or would all lesions eventually become malignant? White believed that all EAC tumours would eventually become malignant, but he urged clinicians to observe their own cases for a prolonged time and to publish their observations.

Re-examination of White's report now suggests that this publication may be the earliest description of the NBCC syndrome. Of course, the evidence he presented is insufficient for absolute confirmation—none of the stigmata such as pits of the hands and feet, ectopic calcification, skeletal defects or jaw cysts were described (Howell, Anderson & McClendon, 1964), nor would such descriptions necessarily be expected. If symptomatic, these defects might have been considered irrelevant to a report of the skin lesions or, if asymptomatic, they would not have been detected because X-rays were not discovered by Roentgen until 1 year later, 1895.

We now know that the clinical behaviour of the tumours described by White was not characteristic of EAC but paralleled that of NBCC: the tumours began to appear relatively early in life, slowly increased in number and size, were found principally on the forehead, nose and eyelids, and, as shown by the patient's photograph, became locally aggressive, invasive, and destructive. This clinical behaviour plus the apparent absence of evidence for any other cause of the multiple rodent ulcers strongly supports a retrospective diagnosis of the NBCC syndrome.

White's diagnosis of EAC, based on Bowen's histological study, can be explained by the histological difficulty in differentiating EAC and NBCC, a difficulty certainly not realized at the turn of the century and not fully appreciated even today. It was this histological similarity and the subsequent confusion about the benign nature of EAC that postponed recognition of these unusual cases as examples of the NBCC syndrome.

During the same year a second puzzling case was reported. Jarisch (1894) described several cases that he had diagnosed as EAC, including one case (Case 2) that had been difficult to classify. Clinically, the tumours on the face of the 22-year-old man were multiple rodent ulcers, but histologically they resembled EAC. Jarisch chose the latter diagnosis.

Jarisch believed the EAC tumour grew from a hair follicle and could behave either as a benign or as a malignant lesion. Therefore, he proposed two categories, one for non-ulcerous tumours, which he named trichoepithelioma papillosum multiplex, and the other for ulcerated, invasive tumours, which he called trichoepithelioma papillosum rodens.

Jarisch's belief, that a given tumour could follow one of two paths, differed from White's opinion that eventually all EAC tumours would become malignant. Nevertheless, both White and Jarisch presented these unusual cases as variants of EAC and did not recognize the tumours to be unusual. Retrospective appraisal of Jarisch's Case 2 suggests that it, too, represented the NBCC syndrome.

Stelwagon in his book *Treatise on Diseases of the Skin* (1905) described a similar case and expressed the opinion that EAC could be closely related to 'superficial epithelioma or rodent ulcer' and that the unusual cases reported by White (1894), Jarisch (1894) and himself were 'connecting examples'. Stelwagon maintained this opinion in later editions of his book (9th edition, 1921) and thus influenced the thinking of dermatologists for some time.

The controversy escalated when Adamson (1908) reported two cases of multiple rodent ulcer, each with an unusually large number of lesions, and reviewed the unusual cases presented earlier by White (1894), Jarisch (1894) and Stelwagon (1905). Adamson regarded the latter three cases as 'closely related to multiple rodent ulcer, if not actually such' because they resembled two of his cases and noted they had 'many features which distinguish them from the more typical examples of Brooke's affection.'

Although Adamson proposed some clinical and histological characteristics to differentiate these cases from typical EAC, he realized that the two conditions had many features in common. He, too, asked if his cases of multiple rodent ulcers and the cases reported by White, Jarisch and Stelwagon might be 'a connecting link between the common rodent ulcer and the multiple benign growths described by Brooke?'

Adamson found that when he assessed the various cases as a series, the differences did not seem so great. He suggested that study of the relations among these cases might have 'a bearing on the question as to what is the essential difference between a benign and a malignant epitheliomatous growth.'

As time passed, the distinctions between the categories proposed by Adamson broke down in practice. Adamson (1914) himself later stated, when commenting on a case that Little (1914) had presented as EAC with ulceration, 'I think this is a case of multiple rodent ulcer, or multiple benign cystic epithelioma (EAC), which I regard as aetiologically the same condition.'

Retrospective appraisal of Adamson's cases strongly suggests that the tumours in the first case were NBCC because of the early age at onset, the location about the eyes, nose and forehead, the large number of lesions (20 or more), rapid growth and ulceration of the lesions and absence of other known causes of multiple skin cancer. The tumours in the second case were probably multiple rodent ulcers (13 lesions) because they appeared at the age of 67, which is later than expected for NBCC.

Hartzell (1908) also reported a case of EAC with ulceration in a 29-year-old woman. Numerous small tumours, which had first appeared several years earlier, were scattered over her face and neck. Several were crusted and 'upon the end of her nose was an oval, rather deep ulcer ... with an elevated, rounded border indistinguishable from the ordinary epitheliomatous ulcer.' Hartzell then repeated White's concern about considering EAC benign and went on to state that 'since, in a fair proportion of cases, destructive ulceration has been observed, as well as a tendency to recurrence after removal, the affection can no longer be regarded as a benign one.' In retrospect, Hartzell's case certainly was not EAC but either multiple rodent ulcers or, perhaps, the NBCC syndrome.

In 1922, Savatard of Manchester reviewed the cases of EAC under his care and described a 15-year-old girl who had had multiple small tumours on her face and neck for 2 years. One tumour on her right lower eyelid had ulcerated; clinically it was indistinguishable from a rodent ulcer and histologically it was malignant. Savatard's description of this case is consistent with our understanding of NBCC, and today, this patient would be examined for stigmata of the NBCC syndrome.

Savatard recommended treatment of the multiple tumours of EAC with X-rays, a recommendation that, unfortunately, was followed indiscriminately for a number of years.

Savatard believed that tumours of EAC never involuted spontaneously but could, on rare occasions, become malignant. Rather than classifying these unusual cases as either EAC with transformation or rodent ulcers, Savatard offered an alternative. He suggested that in some patients benign (EAC) and malignant tumours (rodent ulcer) developed and existed independently.

Summerill & Hutton (1932) revived Adamson's initial concept that these ususual cases were, in fact, basal cell carcinoma, not EAC, which they believed was benign. According to Summerill and Hutton, several of the earlier cases presented as EAC with ulceration had been subsequently reviewed by other dermatologists, who had concluded they were cases of basal cell carcinoma.

Summerill and Hutton proposed that confusion had arisen because early stages of basal cell carcinoma could appear histologically similar to EAC. Since basal cell carcinoma is a slow-growing, low-grade malignant lesion that shares the same anatomical distribution and cellular origin with EAC, clinical and microscopic differentiation of the two conditions could be difficult. This similarity, they believed, had led clinicians to diagnose early stages of basal cell carcinoma as EAC and then, when malignant characteristics appeared, to conclude that the benign lesions had been transformed. They also emphasized that no cases had been presented as basal cell epithelioma (rodent ulcer) that actually were EAC.

The first hint that these enigmatic tumors might be neither EAC nor one of the known varieties of rodent ulcer is found in a fascinating report by Nomland (1932). He described a case of multiple basal cell epithelioma that originated from congenital pigmented basal cell naevi in a 38-year-old woman. At the time of his report, more than 50 'pigmented naevi' were scattered over the patient's face and neck; flat, hairless, dark brown lesions of this type had been present since birth. About 20 other 'naevi' on her eyelids had recently enlarged to resemble lesions of molluscum contagiosum, and two frank epitheliomas, one on the right side of her nose and the other beneath her right eye, had developed from pre-existing 'naevi.'

Nomland considered these tumours to be unique because they were neither EAC nor pigmented naevi, which they resembled clinically. He believed this case demonstrated transformation of benign tumours into malignant lesions and coined a term for these unique tumours. 'Because of this lack of activity in all three (*sic*) [these] lesions, the origin from the basal cell layer of the epidermis, the dominant basal cell type of cell, the presence of pigment cells and the essential similarity, they were considered as quiescent basal cell nevi'.

Nomland could find no previous reports of identical cases, but he did note that his case was similar to the cases reported years earlier by Adamson (1908), except in Adamson's cases the tumours were not pigmented and the ulcerated lesions had not developed from pre-existing 'naevi.' Nomland's case was, in fact, an example of the NBCC syndrome; the lesions did not become malignant, but were always NBCC that became aggressive with time. Nomland lived to know the full story of his patient's condition and the other features of the syndrome, which he found in cases he later observed (personal communication).

The relation of Nomland's basal cell naevi to the puzzling cases of ulcerated EAC remained unrecognized until 1938 when the concept that a few EAC tumours could occasionally become malignant received support from one of the masters of dermal pathology, Pinkus (1938). In a review of some uncommon skin tumours, he described the curious case of a 57-year-old man who had approximately 30 small 'moles' distributed over the centre of his face and some larger lesions that were crusted or had the 'rolled, pearly edges of rodent ulcer.' The tumours had not attracted the patient's attention until they had started to grow and ulcerate during the 2 years before he saw Pinkus. Pinkus reported that at examination most of the tumours ranged from 'millet-seed to split-pea size,' and 'looked like moderately pigmented or nonpigmented soft moles,' although none of the lesions contained hair.

Biopsy of the three rodent ulcers showed 'the typical structure of invading basal cell carcinoma' and biopsy of 12 smaller lesions 'exhibited the typical picture of epithelioma adenoides cysticum.' Calcified globules were found both in the epithelial strands and free in the connective tissue. True bone formation was encountered in two instances.

Pinkus summarized this case with the opinion: 'Convincing signs were found that these tumours had originated by transformation from the benign growths. Thus, we have here a case of typical epithelioma adenoides cysticum with malignant degeneration of some of the lesions.'

Subsequent to the establishment of the NBCC syndrome and at our request, Dr Pinkus generously restudied the slides of this case of EAC. His opinion remained that the tumours were EAC both clinically and histologically and were not NBCC. The age of the patient when the tumours ulcerated (55 years) was later than expected for NBCC and there were fewer ulcerated lesions than in the NBCC syndrome. He did remark that 'none of the tumours was really rodent ulcer or deeply invasive.'... 'When I reviewed the sections now, I was impressed that they were papules with just a superficial central crust, and this I believe is also different from the sometimes very aggressive lesions of the nevoid basal cell carcinoma syndrome' (personal communication).

The next relevant report was published not in the dermatological literature but in the *American Journal of Orthodontics and Oral Surgery*. Straith (1939), an oral surgeon, reported the fascinating cases of three patients, a mother, her 16-year-old son, and her 10-year-old daughter, all of whom had hereditary epidermoid cysts of the jaws.

Straith's report records other anomalies associated with these cysts: in the mother, 'numerous pigmented growths on the face and back' (diagnosed as skin cancers and treated with electrocoagulation and X-rays by a dermatologist) and cysts in the soft tissue of the back and the left patellar region; in the son, cysts of the lower lip and the distal phalanx of the left little finger, a skull that appeared pagetoid and which roentgenograms revealed to be of a hydrocephalic type, and areas of calcification in the falx cerebri and dura; in the daughter, the sella turcica was of average size but tended to be 'roofed.'

Straith pointed out the similarity of all three cases and showed that the condition was hereditary. This was further supported by the fact that the mother's father had had multiple growths on his face and had died at the age of 64 years of an epithelioma on the back of his neck. Straith suggested that development of the epithelial growths was related to endocrine or metabolic changes because the tumours developed during adolescence in both the mother and her son. Development during or shortly after adolescence is, of course, a feature common to many genodermatoses.

Thus, 20 years before the NBCC syndrome would be established, its distinguishing features—multiple basal cell cancers, jaw cysts, ectopic calcification, and cysts of the skin—were described. Yet Straith did not recognize the tumours to be unique and any dermatologists aware of this report apparently did not realize these cases were related to the unusual cases of EAC that had been reported.

Traenkle (1940) presented a histological study specifically directed toward exploring the relation of EAC, trichoepithelioma, and basal cell carcinoma. He found that 'the clinically typical lesions do not necessarily show the histologic picture usually regarded as classic.' Montgomery, in his comments on Traenkle's paper, emphasized 'the difficulty that one encounters histologically in drawing a line between benign and malignant basal cell epitheliomas and the necessity of having both clinical and histologic evidence in a given case.'

Ten years later in his book *Cutaneous Neoplasms*, Norman Paul (1950) described a rare form of skin cancer that he called 'Rodent Ulcer of the Face—Multiple and Naevoid.' The three patients he described had numerous lesions that had appeared abruptly in early adult life and were distributed principally over the face. New crops of tumours appeared periodically and some grew rapidly and soon ulcerated. Other naevoid lesions such as milia were also present. Paul did not classify these cases as EAC because he had 'never seen the slightest tendency of any untreated lesions in my cases [of EAC] to ulcerate.' He classified them as rodent ulcers (basal cell carcinomas) of naevoid origin.

Paul's lucid clinical and histological appraisal of the tumours leaves little doubt that his three patients had NBCC. His description parallels that of NBCC and his patients' numerous lesions occurred in the absence of precancerous keratoses or 'farmer's skin.'

Besides describing three more cases of these unusual tumours, Paul's discussion was of special value because he emphasized the danger of these malignant tumours. He pointed out that these lesions were commonly found about the nasofacial folds and the eyelids and first warned of danger to the eye. Each of his three patients had had an eye destroyed by a tumour or tumours. He stressed the value of frequent observations and immediate treatment of the fresh lesions that continually appeared around the eyes and other strategic sites. Even today these dangers and the value of appropriate and timely treatment are not fully appreciated.

The next year a report finally appeared that drew the attention of dermatologists to the anomalies associated with these unusual skin tumours. This was the case reported by Binkley & Johnson (1951) whose primary purpose was 'to present features of epithelioma adenoides cysticum which have not usually received adequate attention from dermatologists' and was entitled 'Epithelioma Adenoides Cysticum: Basal Cell Nevi, Agenesis of the Corpus Callosum and Dental Cysts.'

The patient, a 30-year-old woman, had, in addition to the skin lesions, a bifid right sixth rib, a necrotic fibroma of the left ovary that was removed at operation at the age of 18, a fatty tumour of the left foot that was excised twice, and multiple follicular cysts of the mandible. One group of these cysts in the right mandible was complicated several years later by fibrosarcoma, which metastasized and led to the patient's death. At autopsy, partial agenesis of the corpus callosum was found. (This feature has not been documented in subsequent cases of the NBCC syndrome.) More than 1000 'slightly raised, hard papules' were widely distributed over the patient's body and several ulcerated lesions were on her face. Clinically, Binkley and Johnson diagnosed some of the lesions to be EAC and others as basal cell carcinoma with ulceration. We now recognize that these skin tumours were NBCC.

The patient's parents and sister had no record of cutaneous or other malformations. On examination, none of her five children had any dental abnormalities, but one daughter did have skin lesions that, based on a histological study, were reported as EAC.

The idea that these skin tumours might be 'a single feature of a generalized disorder' began to emerge with this publication. Binkley and Johnson noted a parallel with the epiloia syndrome (tuberous sclerosis) in which the cutaneous anomaly is one of many developmental defects affecting several systems.

Binkley and Johnson were unaware that EAC was not consistently associated with developmental defects and they suggested that all patients with EAC should be examined for associated multisystem developmental anomalies. Nevertheless, their expressed hope that their paper 'might contribute to our knowledge of the general group of hereditary diseases in which cutaneous tumours are a single feature of a generalized disorder' has now been admirably fulfilled.

Alerted by Binkley and Johnson's article, Gross (1953) reported a similar case in the *Journal of Oral Surgery*. His patient, a 9-year-old boy, had follicular cysts of the maxilla and mandible, synosteosis of the left first rib, bifid left and right sixth ribs and cutaneous tumours. A dermatologist diagnosed the latter as EAC based on a histological study. The description of this case parallels the now recognized features of the NBCC syndrome so that there is no question but that this case, too, was an example of the syndrome.

In 1957, Lund selected the case of Binkley and Johnson's patient to illustrate 'multiple basal cell tumors (both trichoepitheliomas and basal cell carcinomas)' and that of the patient's daughter to show 'multiple trichoepitheliomas (basal cell nevi).' Lund's comments about a histological section from one of the daughter's tumours reflected the opinion of most experts at that time, that EAC could transform into multiple rodent ulcers. 'The distinction between basal cell epithelioma and trichoepithelioma [EAC] is only one of degree. Localization by connective tissue, lack of bulky growth of individual cell clusters, and scanty mitotic figures correlate with the indolent clinical course. The eventual outcome in this case is problematic. It is likely that some of these lesions will become more aggressive.'

It was this accumulation of data—derived, interpreted, debated and reinterpreted—that constituted the body of information that led Howell & Caro (1959) to establish the NBCC syndrome. They perceived a close similarity between two of their cases (Cases 1 and 2) and the case described by Binkley and Johnson. Howell and Caro recognized that the cutaneous tumours associated with developmental anomalies were not EAC with malignant transformation, as previously reported. They proposed that the tumours were actually a unique type of multiple rodent ulcer with a predilection for the central region of the face. They called these tumours 'basal cell naevi,' temporarily retaining Nomland's term. They later preferred the term 'naevoid basal cell carcinoma', since it called attention to the potential danger of the tumours and avoided the term 'naevi,' which was confusing to some.

Howell and Caro drew attention to the striking discrepancy between the histological and clinical appearance of the tumours. The tumours of EAC and NBCC were histologically similar, but the two conditions could be distinguished by their different biological behaviour; tumours of EAC remained benign whereas NBCC became highly destructive with time.

This difference in tumour behaviour allowed Howell and Caro to separate these lesions from those of EAC and to identify them as unique. Recognition that they were associated with a constant constellation of developmental defects led Howell and Caro to establish for the first time a syndrome composed of multiple NBCC (basal cell naevi), multiple cysts of the jaw, developmental anomalies of the skeleton and other frequently encountered anomalies.

They emphasized the potential danger of these tumours to the eyelids, nose, and the centre of the face and reinforced Paul's (1950) earlier warning of danger to the eyes. They advocated conservative surgical treatment of all tumours of the eyelids, nose and neighbouring areas and a programme of regular, frequent follow-up examinations to detect and 'harvest' active lesions and any lesions involving strategic sites. Because of the long-term ill effects of radiation administered to large areas of the skin, they discouraged radiation therapy except for a lesion difficult to treat otherwise.

Having established that these tumours were not EAC, Howell and Caro were able to offer an explanation to resolve the controversy about the propensity of EAC to become malignant. And they were able to co-ordinate and reinterpret data from diverse areas of study that had not been recognized to be related either to the puzzling cases of EAC or, in some instances, to each other: the cases of multiple rodent ulcers described by Paul (1950), of basal cell naevi by Nomland (1932) and of skin tumours and cysts of the jaws by Straith (1939), Binkley & Johnson (1951) and Gross (1953).

The following year, Gorlin & Goltz (1960) reported two cases of NBCC associated with jaw cysts and, in one case, also with a bifid rib. This confirmed the concept that these features constituted a syndrome as proposed by Howell and Caro.

Subsequent reports of many other cases describing developmental anomalies, other stigmata and features associated with these carcinomas (for references, see Howell & Anderson, 1976) have provided a nucleus of information necessary to discern just which features are found consistently. At the present time, the NBCC syndrome can usually be diagnosed on the presence of multiple rodent ulcers that appear unusually early in the patient's life, jaw cysts, pits of the hands and/or feet, ectopic calcification, and developmental skeletal defects. A detailed review (Howell & Anderson, 1976) includes discussion of the natural history and management of the cutaneous tumours and the jaw cysts, associated defects that rarely occur, and the genetic features of the disease.

Besides increasing our knowledge and understanding of this syndrome, recent reports have engendered worldwide interest in and curiosity about the syndrome even to the extent that the NBCC syndrome was diagnosed from bone anomalies in two ancient Egyptian skeletons (Satinoff & Wells, 1969).

To return to the original issue, can EAC tumours transform into *multiple* rodent ulcers? Based on our experience and the data in the literature, we would answer no. Besides Pinkus' report (1938), we have found only eight reported cases of documented EAC with ulcerated lesions that were reported to be or to resemble basal cell carcinoma and that occurred among the 120 EAC patients described in the literature (Savatard, 1938; Joseph, 1956; Ziprkowski & Schewach-Millet, 1967; Welch, Wells & Kerr, 1968; Wolken, Spivey & Blodi, 1969; Epinette & Bennett, 1972; Gottschalk, Graham & Aston, 1974; Rasmussen, 1975). In one case (Welch *et al.*, 1968), ulceration may have resulted from treatment with X-rays; in the other seven cases, ulceration seems to have been spontaneous. But, in none of these cases were multiple (five or more) rodent ulcers reported.

Gray & Helwig (1963) reported that although the three ulcerated lesions observed in their study of 50 patients with EAC resembled basal cell carcinomas clinically, none was malignant histologically. Perhaps the ulcerated lesions in the eight cases were likewise not malignant. Thus, our estimate, based on the literature, that ulceration occurs in 5% of the cases of EAC almost certainly includes cases of non-malignant ulcerated lesions. This estimate, then, must be considered only an upper limit of the transformation frequency.

The idea that EAC does not transform into rodent ulcers is further supported by the observation of Fliegelman & Kruse (1948) that multiple rodent ulcers were not observed in one family member and EAC in another member of the same family. Rasmussen (1975) did report that a brother of his patient also had multiple ulcerating lesions of the face that were diagnosed as basal cell epithelioma, yet the patient was described as having only one ulcerated lesion which was histologically interpreted as trichoepithelioma.

That some of these cases are truly EAC that have transformed to basal cell carcinoma is likely, but these few case reports emphasize that transformation occurs infrequently and that ulceration does not necessarily mean a malignant lesion. No report of multiple transformations of EAC has been published and we have never observed this clinically.

This opinion does not rule out the chiefly theoretical possibility that multiple rodent ulcers may develop independently in patients with EAC. Indeed, a patient with EAC, similar to an individual without EAC, can be susceptible to carcinogens. Arsenic, tars, and pitch can cause multiple rodent ulcers in the susceptible subject as can old age, albinism, xeroderma pigmentosum and chronic skin damage from ultraviolet light. In addition, some patients with EAC have an increased chance of developing rodent ulcers because they have been exposed to high doses of X-rays. For a number of years radiation therapy was used to improve the cosmetic appearance by flattening the multiple EAC tumours of the face.

But any cases that appear to be spontaneous transformation of EAC tumours into multiple rodent ulcers must be evaluated very carefully. The tumours may be NBCC and the patient may have the NBCC syndrome. Early diagnosis and timely treatment are of critical importance in the management of this fascinating genetic condition with dangerous tumours.

REFERENCES

Adamson, H.G. (1908) Two cases of multiple rodent ulcers with a note on the possible relationship between multiple rodent ulcer and epithelioma adenoides cysticum of Brooke. *Lancet*, **i**, 1133.

Adamson, H.G. (1914) Multiple rodent ulcer (?) or epithelioma adenoides cysticum. *Proceedings of the Royal Society of Medicine* (Section on Dermatology), **8**, 48.

Binkley, G.W. & Johnson, H.H., Jr. (1951) Epithelioma adenoides cysticum: Basal cell nevi, agenesis of the corpus callosum and dental cysts. *Archives of Dermatology and Syphilology*, **63**, 73.

Brooke, H.G. (1892) Epithelioma adenoides cysticum. *British Journal of Dermatology*, **4**, 269.

Epinette, W.W. & Bennett, J.E. (1972) Anaplastic 'pilar' carcinoma arising in trichoepithelioma. *Cutis*, **10**, 446.

Flieghelman, M.T. & Kruse, W.T. (1948) Hereditary multiple benign cystic epithelioma. *Journal of Investigative Dermatology*, **11**, 189.

Fordyce, J.A. (1892) Multiple benign cystic epithelioma of the skin. *Journal of Cutaneous and Genito-Urinary Disease*, **10**, 459.

Gorlin, R.J. & Goltz, R.W. (1960) Multiple nevoid basal cell epithelioma, jaw cysts, and bifid rib: A syndrome. *New England Journal of Medicine*, **262**, 908.

Gottschalk, H.R., Graham, J.H. & Aston, E.E. (1974) Dermal eccrine cylindroma, epithelioma adenoides cysticum of Brooke, and eccrine spiradenoma. *Archives of Dermatology*, **110**, 473.

Gray, H.R. & Helwig, E.B. (1963) Epithelioma adenoides cysticum and solitary trichoepithelioma. *Archives of Dermatology*, **87**, 102.

Gross, P.P. (1953) Epithelioma adenoides cysticum with follicular cysts of maxilla and mandible. *Journal of Oral Surgery*, **11**, 160.

Hartzell, M.B. (1908) A further contribution to the study of benign cystic epithelioma. *Journal of Medical Research*, **18**, 159.

Howell, J.B. & Caro, M.R. (1959) The basal cell nevus. *Archives of Dermatology*, **79**, 67.

Howell, J.B., Anderson, D.E. & McClendon, J.L. (1964) The basal cell nevus syndrome. *Journal of the American Medical Association*, **190**, 274.

Howell, J.B. & Anderson, D.E. (1976) The nevoid basal cell carcinoma syndrome. In: *Cancer of the Skin: Biology–Diagnosis–Management* (Ed. by R.Andrade, S.L.Gumport, G.L.Popkin *et al.*). W.B.Saunders, Philadelphia.

Jarisch, A. (1894) Zur Lehre von den Hautgeschwulsten. *Archiv für Dermatologie und Syphilologie*, **28**, 163.

Joseph, H.L. (1956) Epithelioma adenoides cysticum with basal cell carcinoma. *Archives of Dermatology*, **74**, 113.

Little, E.G.G. (1914) A note on two cases of epithelioma adenoides cysticum (Brooke), Trichoepithelioma papulosum rodens (Jarisch). *British Journal of Dermatology*, **26**, 173.

Lund, H.R. (1957) Tumors of the skin. In: *National Research Council Committee on Pathology: Atlas of Tumor Pathology*, p. 14. Armed Forces Institute of Pathology, Washington, D.C.

Nomland, R. (1932) Multiple basal cell epitheliomas originating from congenital pigmented basal cell naevi. *Archives of Dermatology and Syphilology*, **25**, 1002.

Paul, C.N. (1950) *Cutaneous Neoplasms*, p. 46. H.K.Lewis, London.

Pinkus, H. (1938) Some uncommon skin tumors. *Journal of the Michigan Medical Society*, **27**, 533.

Rasmussen, J.E. (1975) A syndrome of trichoepitheliomas, milia, and cylindromas. *Archives of Dermatology*, **111**, 610.

SATINOFF, M.I. & WELLS, C. (1969) Multiple basal cell naevus in ancient Egypt. *Medical History*, **13**, 394.

SAVATARD, L. (1922) Epithelioma adenoides cysticum. *British Journal of Dermatology*, **34**, 381.

SAVATARD, L. (1938) Epithelioma adenoides cysticum. *British Journal of Dermatology*, **50**, 333.

STELWAGON, H.W. (1905) *Treatise on Diseases of the Skin*, 4th edn, p. 618. W.B.Saunders, Philadelphia.

STELWAGON, H.W. & GASKILL, H.K. (1921) *Treatise on Diseases of the Skin*, 9th edn, p. 684. W.B.Saunders, Philadelphia.

STRAITH, F.E. (1939) Hereditary epidermoid cysts of the jaws. *American Journal of Orthodontics and Oral Surgery*, **25**, 673.

SUMMERILL, F. & HUTTON, J.G. (1932) Multiple benign cystic epithelioma (epithelioma adenoides cysticum). *Archives of Dermatology and Syphilology*, **26**, 854.

TRAENKLE, H.L. (1940) Epithelioma adenoides cysticum, trichoepithelioma and basal cell cancer: Relationship between these diseases, as shown by histologic studies of multiple benign cystic epithelioma. *Archives of Dermatology and Syphilology*, **42**, 822.

WELCH, J.P., WELLS, R.S. & KERR, C.B. (1968) Ancell-Spiegler cylindromas (turban tumors) and Brooke-Fordyce trichoepitheliomas: Evidence for a single genetic entity. *Journal of Medical Genetics*, **5**, 29.

WHITE, J.C. (1894) Multiple benign cystic epithelioma. *Journal of Cutaneous and Genito-Urinary Diseases*, **12**, 477.

WOLKEN, S.H., SPIVEY, B.E. & BLODI, F.C. (1969) Hereditary adenoid cystic epithelioma. Brooke's tumor. *American Journal of Ophthalmology*, **68**, 26.

ZIPRKOWSKI, L. & SCHEWACH-MILLET, M. (1967) Hereditary epidermis tumor. 1. Multiple trichoepithelioma. *Proceedings of the Telhashomer Hospital*, **6**, 70.

British Journal of Dermatology (1976) **95**, 243.

Histopathological spectrum of erythema multiforme

T.R.BEDI* AND HERMANN PINKUS

Department of Dermatology, Wayne State University School of Medicine, Detroit, and the
Detroit General Hospital

Accepted for publication 5 January 1976

SUMMARY

Lesions of erythema multiforme from seventy-five patients have been studied histologically. In addition to peculiar intercellular epidermal oedema, subepidermal separation and a lymphohistiocytic inflammatory infiltrate in the papillary dermis, epidermal cell necrosis was observed in a variable percentage of the lesions. While dermal disturbance was a predominant finding in the macular lesions, focal or generalized keratinocytic necrosis was seen in the macular, papular, bullous and iris lesions in that order of frequency. Significant numbers of eosinophils were present in the inflammatory infiltrate in 60% of the bullous and 28% of the macular lesions. Our findings suggest that erythema multiforme represents a tissue reaction with spectral expression, one end presenting as a predominantly dermal disturbance and the other merging into the adult type of toxic epidermal necrolysis. Yet, the histological features remain sufficiently characteristic for differentiation from other erythematous and vesiculobullous eruptions.

Erythema multiforme is a reaction pattern with varied clinical features. Macules, papules, vesicles, bullae and iris lesions are all known to occur. Although individual patients may have only one type of lesion, the occurrence of different morphological forms of lesions in the same patient is more frequent. In the past, erythema multiforme has been regarded as a primary dermal disturbance histologically. However, recent observations (Ackerman, Penneys & Clark, 1971; Prutkin & Fellner, 1971; Orfanos, Schaumburg-Lever & Lever, 1974) suggest that both epidermis and dermis are primarily affected. We attempted to make our own observations from lesions of erythema multiforme seen in seventy-five patients, in whom clinical and histological features supported the diagnosis.

MATERIALS AND METHODS

Skin biopsy specimens from 75 cases of erythema multiforme were studied. In each case the duration

---

* Senior Resident, Department of Dermatology, All India Institute of Medical Sciences, New Delhi, India and Fellow, Dermatology, Wayne State University School of Medicine, Detroit, Michigan, U.S.A.

This work was supported by the Foundation for International Dermatologic Education and by the Detroit General Hospital Research Corporation.

Reprint requests to: Hermann Pinkus, M.D., Department of Dermatology, Wayne State University School of Medicine, 540 East Canfield Ave., Detroit, Michigan 48201, U.S.A.

TABLE 1. Histopathological features of different types of lesions of erythema multiforme

Histopathological findings	Type of lesions				Total number 75
	Macular 29	Papular 21	Iris 5	Bullous 20	
Epidermal oedema					
Intercellular	21 (72)	21 (100)	5 (100)	20 (100)	67 (90)
Intracellular	8 (28)	16 (76)	5 (100)	13 (65)	42 (56)
Epidermal necrosis					
Focal	6 (21)	11 (52)	5 (100)	14 (70)	36 (48)
Generalized	0	4 (2)	3 (60)	11 (55)	18 (24)
Papillary oedema	29 (100)	21 (100)	5 (100)	20 (100)	75 (100)
Separation					
Subepidermal	1 (3)	10 (48)	5 (100)	15 (75)	31 (41)
Intra-epidermal	1 (3)	9 (43)	2 (40)	7 (35)	19 (25)
Dermal inflammation					
Lymphohistiocytic	27 (93)	21 (100)	5 (100)	20 (100)	73 (97)
Lymphocytic chiefly	2 (7)	0	0	0	2 (3)
Eosinophils present in significant number	8 (28)	8 (4)	0	12 (60)	28 (37)
Site of inflammation					
Superficial vascular	29 (100)	21 (100)	5 (100)	20 (100)	75 (100)
Extending deep	4 (14)	2 (10)	0	0	6 (8)

The figures in parentheses represent the percentages.

of the lesion biopsied and the total duration of the disease were recorded. Any history of concomitant or preceding infection, drug intake or any other suggestive aetiology was taken into account. The biopsy lesions included macules (29), papules (21), iris lesions (5) and bullae (20). The skin specimens were processed in the routine manner; 10 μm thick sections were cut and stained with haematoxylin–eosin and acid orcein–Giemsa.

Histopathology

The histopathological changes seen are summarized in Table 1. These findings with their clinical correlation are presented as follows.

Epidermal oedema. Ninety percent of the lesions showed inter-cellular oedema. Most marked in papular, iris and bullous lesions, the oedema characteristically was seen to stretch the epidermal cells vertically suggesting transudation of fluid in a stream from the vessels (Fig. 1). A variable degree of intracellular oedema was also observed in 56% of the lesions. Presenting as empty halos around the nuclei of the epidermal cells, it was rather a mild and relatively infrequent finding in the macular lesions. All iris lesions and a majority of papular and bullous lesions showed considerable intracellular oedema.

Intra-epidermal vesiculation was seen in 19 lesions (Fig. 2). Of these, five showed subcorneal vesicles. Two-thirds of all lesions of erythema multiforme showed a variable degree of exocytosis into

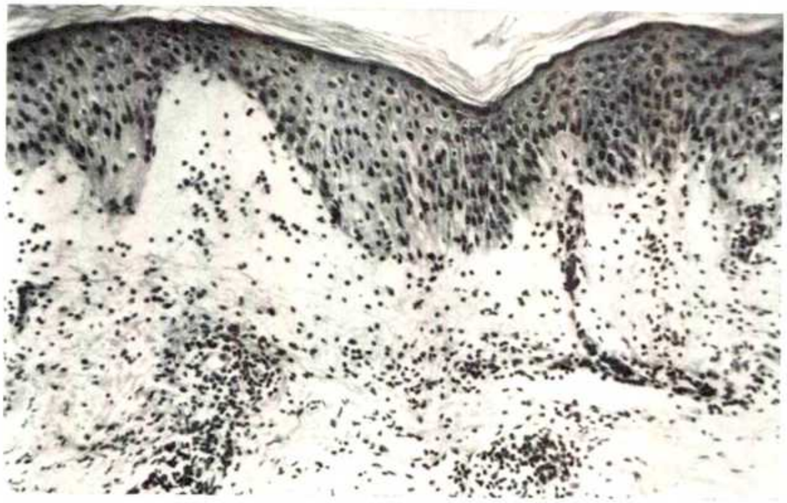


FIGURE 1. Section from a papular lesion of erythema multiforme exhibiting characteristic epidermal oedema stretching the cells vertically, subepidermal vesiculation and dermal inflammation (H & E, ×60).

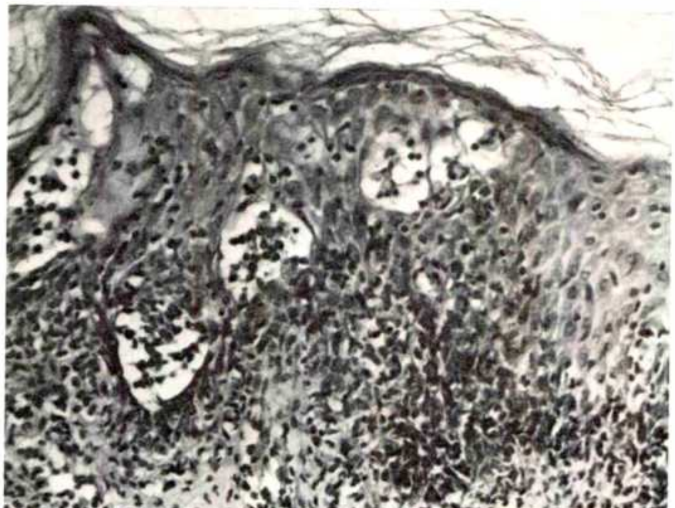


FIGURE 2. Intra-epidermal vesiculation and exocytosis with a normal stratum corneum. Note characteristic epidermal oedema on the far right (H & E, ×180).

the epidermis. The migrating cells being mostly a mixture of polymorphs and round cells were often seen to concentrate within the intra-epidermal vesicles.

Epidermal necrosis. More than two-thirds of the lesions showed epidermal cell necrosis of either focal or generalized nature. Many keratinocytes appeared pyknotic or necrotic (Fig. 3). Only six of the twenty-nine macular lesions showed necrotic foci in the epidermis histologically. While focal areas

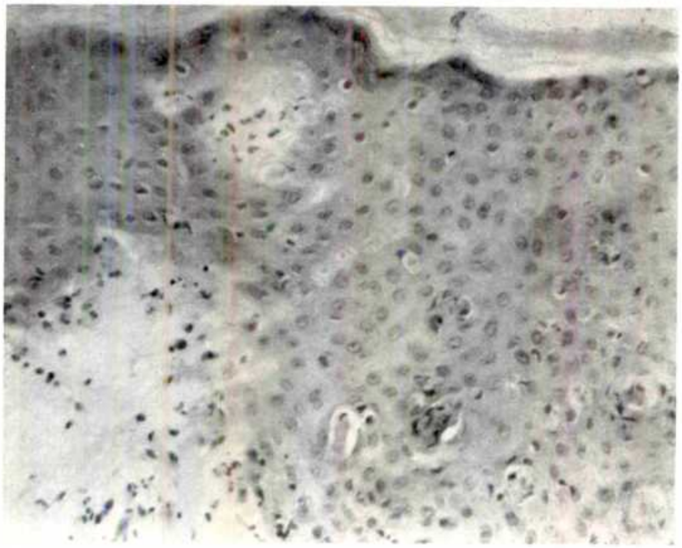


FIGURE 3. Numerous foci of acidophilic keratinocytes observed in the epidermis overlying the dermal bulla of erythema multiforme (H & E, × 225).

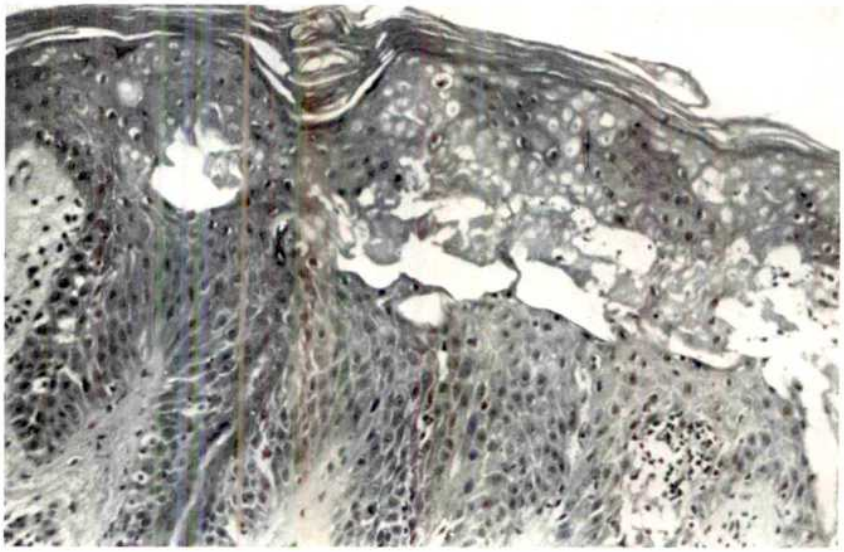


FIGURE 4. Papular lesion of erythema multiforme showing extensive epidermal necrosis of the upper layers. Stratum corneum is unaffected and sweat duct remains preserved (H & E, × 180).

of necrosis were also present in eleven of the twenty-one papular lesions, four of these showed generalized epidermal necrosis (Fig. 4). All iris lesions and more than half of the bullous lesions showed signs of epidermal necrosis. In the iris lesions the whole epidermis overlying the oedematous papillary dermis appeared completely necrotic, composed of either cells with pyknotic nuclei or amorphous acidophilic bodies (Fig. 5). Bullous lesions showed less intense necrosis with more pronounced subepidermal oedema (Fig. 6). However, focal areas of necrosis of the basal keratinocytes were

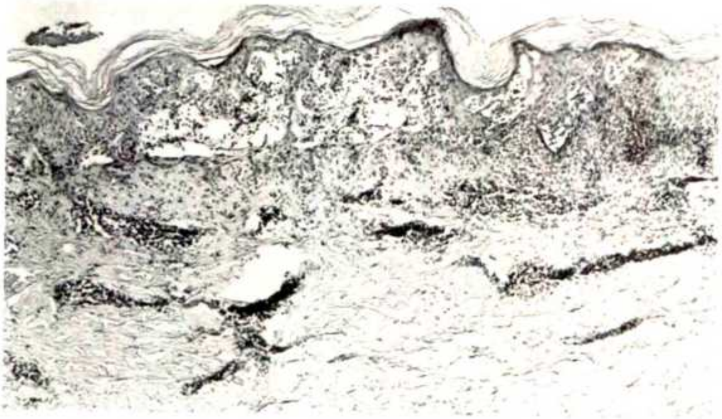


FIGURE 5. Iris lesion exhibiting central epidermal necrosis with vasodilation and dermal inflammation in a coat-sleeve fashion (H & E, × 60).

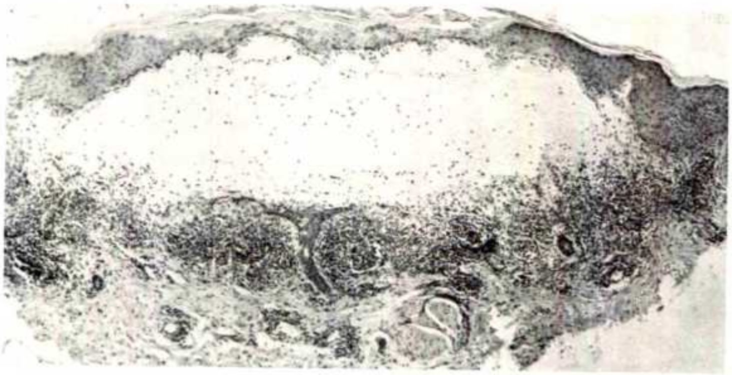


FIGURE 6. Bullous lesion showing pronounced subepidermal vesiculation and extensive necrosis of the overlying epidermis. At the base of the bulla is seen a part of the sweat duct unaffected by the perivascular dermal infiltrate (H & E, × 45).

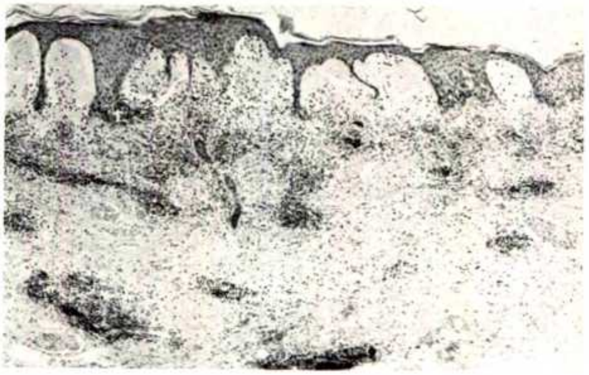


FIGURE 7. Low power view of a section from a papular lesion of erythema multiforme depicting a predominantly dermal disturbance in the form of subepidermal bullous oedema and lymphohistiocytic inflammatory infiltrate surrounding the superficial vascular plexus in the dermis. Intercellular oedema of the epidermis is also present (H & E, × 45).

noticeable in 48% of all types of lesions. The epidermal cell necrosis was mostly interpreted as an early event without any evidence of regeneration, parakeratosis or granular layer involvement. The stratum corneum appeared unaffected by the underlying epidermal changes.

Papillary oedema and dermo–epidermal separation. All lesions presented a variable degree of papillary oedema giving a characteristic vacant look to the immediate subepidermal zone. In about half the lesions the papillary oedema was so intense as to produce subepidermal separation (Fig. 7). Almost all bullous and iris lesions showed massive subepidermal oedema producing separation, and the overlying epidermis in these lesions presented generalized or focal necrosis. A majority of papular lesions also showed papillary oedema, but usually less pronounced. The basement membrane in most instances could be made out at the roof of the separation bullae. The presence of melanin in cells of the basal layer facilitated this observation.

Dermal inflammatory infiltrate. A variable degree of inflammation was seen in the dermis. Although almost all lesions showed an infiltrate around the superficial vascular plexus, the infiltrate extended deep in sections from six lesions (4 macular and 2 papular). In 97% of the lesions the infiltrate was composed of a mixture of lymphocytes and histiocytes. In only two macular lesions was the infiltrate chiefly lymphocytic. The inflammatory infiltrate was either seen along the vascular channels in a coat sleeve fashion (Fig. 8) or was so pronounced as to occupy the entire superficial dermis yet maintaining its relation to the superficial vascular plexus. In two iris and two bullous lesions showing pronounced inflammation histologically, extravasated red blood cells were found in the subepidermal zone. Significant numbers of eosinophils were observed in 37% of the lesions. While a variable proportion of macular, papular or bullous lesions (Table 1) contained eosinophils in the infiltrate, none of the iris lesions showed significant tissue eosinophilia. Apart from occasional dilatation and endothelial swelling, no appreciable vascular changes were found. Signs of vasculitis were absent. Cutaneous appendages were uninvolved.

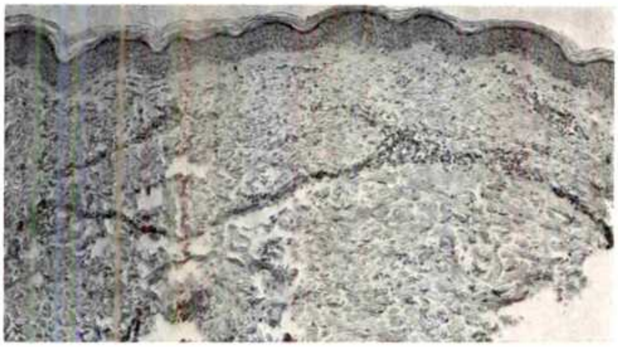


FIGURE 8. Coat-sleeve like perivascular infiltrate composed chiefly of mononuclear cells and a mild epidermal oedema seen in a section from macular lesion of erythema multiforme (H & E, × 45).

DISCUSSION

The diagnosis of erythema multiforme is primarily a clinical one (Lyell, 1971; Porter, 1972; Champion, 1972) and is based in part on the variability of the lesions and in part on the characteristic evolution to 'iris' configuration of at least some of them. There are, however, many lesions that do not progress beyond the maculo-papular stage. Histological examination is called for in order to differentiate less typical cases either from other forms of bullous eruptions (dermatitis herpetiformis, pemphigoid) or from other erythematous dermatoses (lupus erythematosus, drug eruptions, vasculitis). Recent histopathological studies (MacVicar, Graham & Burgoon, 1963; Ackerman *et al.*, 1971; Orfanos *et al.*, 1974) put emphasis on several features in both dermis and epidermis as being diagnostic of erythema multiforme, without coming to a consensus. We therefore decided to analyze the microscopic features of a fairly large number of cases from our own material and to subdivide them according to the clinical stage of the biopsied lesion.

In our material from seventy-five patients, erythematous macules (29 lesions) showed predominantly dermal disturbance in the form of papillary oedema and a lymphohistiocytic infiltrate surrounding the superficial vascular plexus in a coat sleeve fashion. Eosinophils in significant number were present in the infiltrate in 28% of these lesions. Apart from a commonly observed peculiar intercellular oedema which caused elongation of prickle cells vertical to the skin surface, the epidermis was not appreciably affected in the majority of cases. However, focal aggregates of necrotic keratinocytes were seen in 21% of the macular lesions.

Similar necrotic changes in the epidermis became more frequent and pronounced in the papular lesions so much so that sections from four papules showed generalized epidermal necrosis. Epidermal and dermal oedema was also more conspicuous so as to produce intra-epidermal and subepidermal separation in nineteen of twenty-one cases.

The bullous (20) and the iris (5) lesions revealed more generalized changes. All iris lesions showed epidermal necrosis of varying degree and in 60% of these the epidermis was completely necrotic and indistinguishable from lesions of the adult type of toxic epidermal necrolysis. Irrespective of the site and extent of necrosis of the keratinocytes, the stratum corneum was unaffected. Subepidermal and intra-epidermal vesicles were also seen. The dermal inflammatory infiltrate, however, showed certain differences in the bullous and the iris lesions. While significant numbers of eosinophils were found in the infiltrate of 60% of the bullous lesions, none of the iris lesions showed appreciable numbers of eosinophils.

These findings suggest that different types of lesions of erythema multiforme seen clinically present different yet distinct histological features. Necrotic changes in the epidermis and participation of eosinophils in the dermal infiltrate may be seen in variable degree depending upon the type of lesion biopsied. The classical iris lesions are more likely to show epidermal necrosis. This feature has been emphasized by other workers (Ackerman *et al.*, 1971; Orfanos *et al.*, 1974).

Undoubtedly, the epidermal changes in erythema multiforme appear to be important, especially so in the classical 'iris' lesions where one, at times, may find difficulty in differentiating it from lesions of the adult type of toxic epidermal necrolysis. Clinically, of course, there is little resemblance between the two types of lesions, unless one considers the adult form of Lyell's syndrome as a severe manifestation of erythema multiforme (Lever & Schaumburg-Lever, 1975; Pinkus & Mehregan, 1976). Perhaps, the presence of significant dermal inflammation would favour erythema multiforme. Papillary oedema and dermal inflammation surrounding the superficial vascular plexus remain universal histological findings. Small blood vessels exhibit oedema of endothelial and peripheral cells but evidence of true vasculitis is absent. Exocytosis and intra-epidermal or subcorneal vesiculation are less frequent.

Though a variety of drugs, infectious agents and toxins have been incriminated, in many instances the cause of erythema multiforme remains unknown. A history of drug intake prior to the eruption was available in some of our patients but histological features did not show any correlation with the drug history. A majority of lesions from such patients did not show significant tissue eosinophilia, rather this feature was more common among patients who denied the history of drug intake and were classifiable as 'idiopathic'.

If one was to speculate on the histogenesis of erythema multiforme, it may not be unreasonable to say that initial events involving the dermal vessels possibly lead to transudation of fluid and subsequently of cells. These then manifest as intercellular or intracellular epidermal oedema, papillary oedema, exocytosis and dermal inflammation. More intense changes may, at times, lead to subepidermal haemorrhage. Also, whatever the nature of the 'toxic' factor is, it may simultaneously result in primary epidermal cell necrosis. In other cases, epidermal necrosis appears to be a late secondary feature in a process of severe inflammation. It is remarkable that in most cases the stratum corneum remains unaffected and that there is rarely evidence of the common type of inflammatory epidermal reaction which leads to parakeratosis.

In our experience the histopathology of erythema multiforme presents a varied picture similar to its clinical multiformity. To exclude all lesions not showing a distinct pathological pattern at the dermo-epidermal interface (Ackerman *et al.*, 1971) seems to be too restrictive at the present state of our limited understanding of the aetiopathogenesis of the disease. It seems permissible to regard erythema multiforme as a tissue reaction with spectral expression, one end presenting as a predominantly dermal disturbance and the other merging into the adult type of toxic epidermal necrolysis. In spite of their variability the histological features are sufficiently characteristic for differentiation of erythema multiforme from other vesiculo-bullous eruptions and from erythematous eruptions of toxic erythema, polymorphous light eruption or lupus erythematosus type.

ACKNOWLEDGMENT

We are thankful to Dr Isadore Botvinick for making some of the patients available to us.

REFERENCES

ACKERMAN, A.B., PENNEYS, N.S. & CLARK, W.H. (1971) Erythema multiforme exudativum: Distinctive pathological process. *British Journal of Dermatology*, **84**, 554.

CHAMPION, R.H. (1972) Erythema multiforme. In: *Textbook of Dermatology* (Ed. by A.J. Rook, D.S. Wilkinson and F.J. Ebling), 2nd edn, Vol 1, p. 889. Blackwell Scientific Publications, Oxford.

LEVER, W.F. & SCHAUMBURG-LEVER, G. (1975) Erythema multiforme. In: *Histopathology of the Skin*, 5th edn, p. 122. J.B. Lippincott Co., Philadelphia.

LYELL, A. (1971) Erythema multiforme. In: *Dermatology in General Medicine* (Ed. by T.B. Fitzpatrick *et al.*), p. 598. McGraw-Hill, New York.

MACVICAR, D.N., GRAHAM, J.H. & BURGOON, C.F. (1963) Dermatitis herpetiformis, erythema multiforme and bullous pemphigoid: A comparative histopathological and histochemical study. *Journal of Investigative Dermatology*, **41**, 289.

ORFANOS, C.E., SCHAUMBURG-LEVER, G. & LEVER, W.F. (1974) Dermal and epidermal types of erythema multiforme. *Archives of Dermatology*, **109**, 682.

PINKUS, H. & MEHREGAN, A.H. (1976) *A Guide to Dermatohistopathology*, 2nd edn, p. 160. Appleton-Century-Crofts, New York.

PORTER, P.S. (1972) Erythema multiforme. In: *Clinical Dermatology* (Ed. by J.D. Demis *et al.*), Vol. 2, Unit 7–4, p. 1. Harper and Row, New York.

PRUTKIN, L. & FELLNER, M.J. (1971) Erythema bullosum. *Acta dermato-venereologica*, **51**, 429.

British Journal of Dermatology (1976) **95**, 251.

Histology simulating reticulosis in secondary syphilis

REBECCA E.I.COCHRAN,* JOHN THOMSON, K.A.FLEMING† AND
ALEXANDRA M.M.STRONG

Department of Dermatology, *University of Glasgow, Department of Dermatology, Royal Infirmary, Glasgow,
and †University Department of Pathology, Royal Infirmary, Glasgow

Accepted for publication 5 January 1976

SUMMARY

Three cases of secondary syphilis are described in whom a skin biopsy was performed. In all, the histology bore a striking resemblance to that of malignant lymphoid neoplasm.

The classical cutaneous manifestations of secondary syphilis are usually easy to diagnose, especially when there is a good clinical history and the appropriate investigations are positive. If, however, the eruption is not characteristic, a skin biopsy may be carried out. We should like to report three cases where the histological appearances in isolation would have been misleading.

CASE REPORTS

Case 1

The patient , a 16-year-old girl, was admitted to hospital with a pyrexia and symptoms suggestive of a urinary tract infection. She was noted to have a widespread erythematous papular eruption involving face, trunk and limbs, which had been present for 3 weeks. There were no mucosal lesions. The inguinal lymph nodes were palpable.

Specific serology was as follows: VDRL slide test—positive. Cardiolipin Wassermann reaction—positive. Reiter protein complement fixation test—positive. A skin biopsy was reported as follows: The epidermis is hyper- and parakeratotic with marked epidermotropism of mononuclear lymphoid cells which are forming micro-abscesses in areas. There is inter- and intracellular oedema in the epidermis. In the dermis there is a dense infiltrate which predominantly is perivascular. There is no evidence of a vascular lesion. The infiltrate is composed of histiocytic cells, mononuclear cells, plasma cells and occasional neutrophil polymorphs. Scattered throughout there are large pleomorphic mononuclear cells with hyperchromatic nuclei and prominent nucleoli. Mitotic figures are occasionally found. These findings in the absence of a clinical history are compatible with the diagnosis of cutaneous lymphoid neoplasm.

Case 2

The patient was a 46-year-old woman who had a 6-week history of a widespread rash. It had commenced as a morbilliform rash on her trunk and spread to involve her limbs, palms and soles. In the previous 3 weeks she had felt unwell and latterly complained of a sore throat and headache.

On examination, she had a widespread infiltrated papular rash with some superimposed fine scaling. The rash affected all areas, including palms, soles, scalp and face. There was no loss of hair. There were lesions on the soft palate. Lymph glands were palpable in both axillae.

The presumptive diagnosis was secondary syphilis and serological testing confirmed this: VDRL slide test—positive. Cardiolipin Wassermann reaction—positive. Reiter protein complement fixation test—positive. A skin biopsy was reported as follows: The epidermis shows slight hyperkeratosis with epidermotropism of small mononuclear cells and surrounding inter- and intracellular oedema. The papillary dermis is oedematous and congested and contains numerous small mononuclear cells similar to those seen in the epidermis in the midst of a background of histiocyte cells. In the rest of the dermis there is a perivascular infiltrate of histiocytic cells, mononuclear cells, some of which have large irregular hyperchromatic nuclei, and very occasional plasma cells. Mitotic figures are easily found. The infiltrate extends down to sweat gland level. There is no evidence of a vasculitis. (Fig. 1).

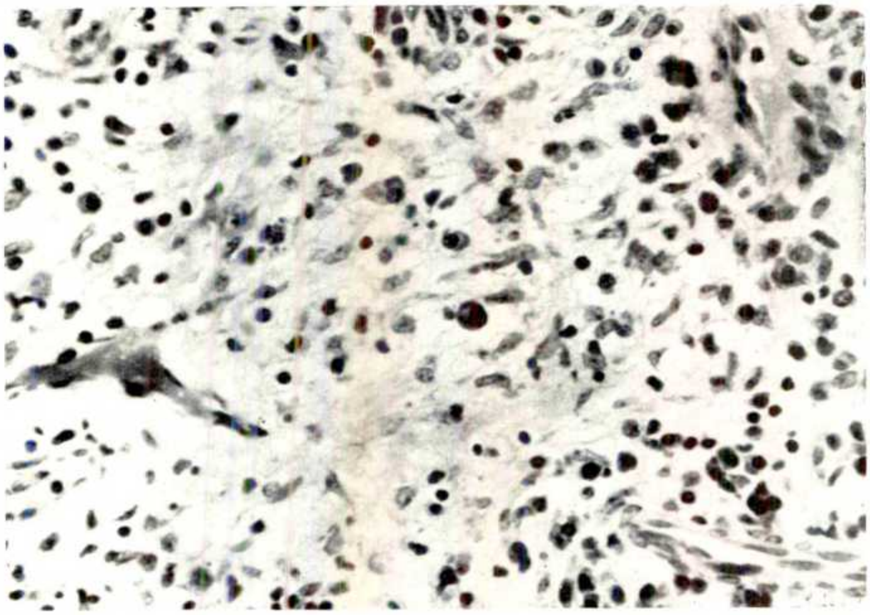


FIGURE 1. Case 2. Shows dermal infiltrate. There are mononuclear cells some with large irregular hyperchromatic nuclei and frequent mitotic figures (H & E, × 1000).

Case 3

A 33-year-old male was referred to the Skin Clinic with a generalized symptomless rash of 4 weeks' duration. He had been treated for ulcerative colitis with Salazopyrin over the previous year and the possibility of a drug eruption had been raised. He admitted to being a passive homosexual and had previously had several perianal abscesses.

On examination, he had a dusky erythematous macular eruption affecting his face, palms, soles and trunk. There were no oral lesions. In the perianal region there were early condylomata lata.

A clinical diagnosis of secondary syphilis was made. Serological tests showed: VDRL slide test—positive. Cardiolipin Wassermann reaction—positive .Reiter protein complement fixation test—positive. A skin biopsy was reported as follows: The epidermis shows focal parakeratosis and mild acanthosis in relation to which there is epidermotropism of lymphocytes and neutrophil polymorphs with intercellular oedema.

In the dermis there is a dense cellular infiltrate which extends down to the fat surrounding sweat glands. The infiltrate is composed predominantly of large cells with pale vesicular central oval nuclei with prominent nucleoli and some foamy cytoplasm. Occasional larger hyperchromatic nuclei are seen and mitotic figures are numerous. There is a diffuse background of plasma cells and occasional melanophages. There is no significant vascular lesion (Fig. 2).

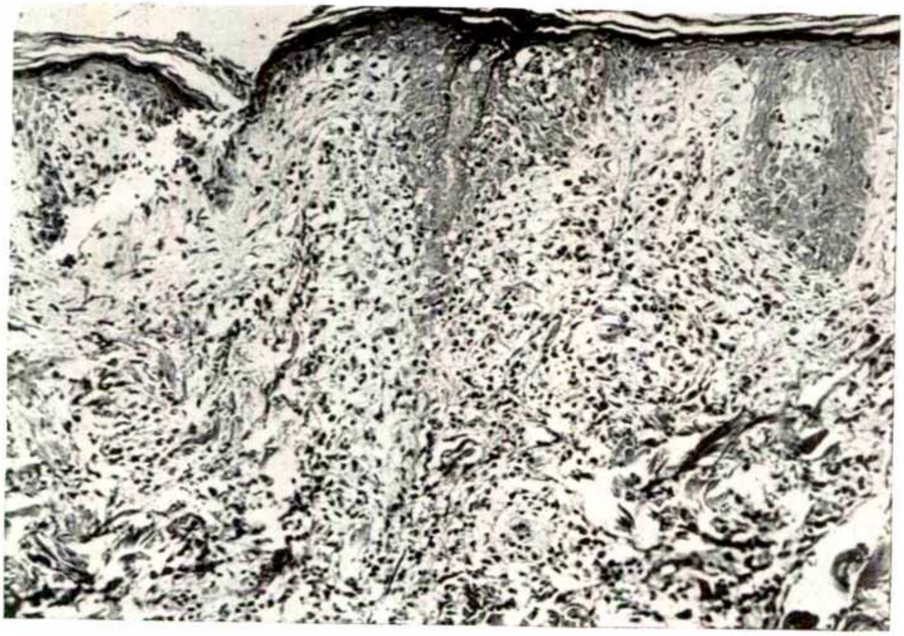


FIGURE 2. Case 3. Shows epidermotropism and a dense dermal infiltrate extending deeply (H & E, × 400).

DISCUSSION

The diagnosis of secondary syphilis was suspected in each case on clinical grounds and confirmed by appropriate laboratory investigation. Thus in our cases the histology while unexpected was not deceiving. All the patients were treated by conventional means and responded satisfactorily.

Histological diagnosis of secondary syphilis from skin biopsies is considered unreliable in view of the fact that the features are so variable and often non-specific (Milne, 1972).

No consistent features were found in a review of sixty skin biopsies in secondary syphilis (Abell, Marks & Wilson Jones, 1973). They noted that prominent endarteritis and marked plasma cell infiltration were less frequent than might be supposed. They suggested that there was early epidermal invasion with inflammatory cells followed later by epidermal psoriasiform hyperplasia. The dermal inflammatory reaction was triangular with the apex deep. A granulomatous inflammation was sometimes seen with predominance of lymphocytes and histiocytes in varying proportions. The same authors (Abell, Marks & Wilson Jones, 1975) in a later more detailed account stressed, however, the value of histopathology in the diagnosis of the unsuspected case despite the variety of histological change. In certain cases, where there was a heavy dermal infiltrate with invasion of mononuclear cells into the epidermis, they felt the appearances could simulate a pre-mycosis or mycosis fungoides. However, large hyperchromatic mononuclear cells were seen in only one biopsy in their series and even then these represented a small proportion of the lympho-histiocytic infiltrate.

Some other workers reviewed twenty-seven biopsies of secondary syphilis in patients whose ages ranged from 4 to 61 years (Jeerapaet & Ackerman, 1973). They found no consistent abnormalities which could be listed and used as a diagnostic aid. Their findings consisted of a superficial and/or deep perivascular infiltrate which might, or might not, contain plasma cells. They concluded that the histology of secondary syphilis was as protean as the clinical manifestations.

However, we feel that there is an important difference between a skin biopsy from which the histopathologist cannot make a definitive diagnosis and one which misleads him into suggesting an erroneous diagnosis. The three cases we describe fall into the latter category and had the correct diagnoses not been established from the clinical examination or appropriate laboratory investigations the wrong therapeutic measures might have been taken with disastrous results.

Our criteria for the histological diagnosis of cutaneous malignant lymphoid neoplasm include a pleomorphic dermal infiltrate consisting of histiocytes, reticulum cells, lymphocytes and plasma cells. Some cells should have hyperchromatic nuclei and others mitotic figures. We feel that the aggregation of this infiltrate around skin appendages, or its occurrence deep to them, is significant. In our present cases these features were present. There was a dense pleomorphic dermal infiltrate. There were numerous large mononuclear cells with large hyperchromatic nuclei, and mitotic figures were present in significant numbers.

The finding of a pleomorphic dermal infiltrate poses certain problems for the histologist if he does not have an accurate clinical history available. The 'pseudo-lymphomas' such as actinic reticuloid and lymphomatoid papulosis would be suggested as possible diagnoses. The possibility of persisting arthropod bite reactions or a response to persistent nodular scabies (Thomson *et al.*, 1974) must not be overlooked. If the infiltrate is arranged in a pattern resembling lymphoid follicles with a rim of eosinophils deep to the lymphocytes, Spiegler-Fendt sarcoid is a possibility. However, these conditions can usually be excluded clinically.

The purpose of this paper is to report the ominous histology we have noted in three cases of secondary syphilis in whom skin biopsies were performed and thus alert clinicians investigating similar patients. We would submit that it is an important diagnostic pitfall. It should be remembered, however, that malignant lymphoid neoplasm and secondary syphilis could co-exist.

ACKNOWLEDGMENTS

We are grateful to Dr Alexander and Dr Lyell, Glasgow Royal Infirmary, for allowing us to report Cases 2 and 3 which were respectively under their care.

REFERENCES

ABELL, E., MARKS, R. & WILSON JONES, E. (1973) Secondary syphilis: a clinico-pathological reappraisal. *British Journal of Dermatology*, **89**, Supplement **9**, 19.

ABELL, E., MARKS, R. & WILSON JONES, E. (1975) Secondary syphilis: a clinico-pathological review. *British Journal of Dermatology*, **93**, 53.

JEERAPAET, P. & ACKERMAN, A.B. (1973) Histologic patterns of secondary syphilis. *Archives of Dermatology*, **107**, 373.

MILNE, J.A. (1972) *An Introduction to the Diagnostic Histopathology of the Skin*. Edward Arnold, London.

THOMSON, J., COCHRANE, T., COCHRAN, R. & MCQUEEN, A. (1974) Histology simulating reticulosis in persistent nodular scabies. *British Journal of Dermatology*, **90**, 421.

British Journal of Dermatology (1976) 95, 255.

Changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats

P.J.HARTOP AND C.PROTTEY

Environmental Safety Division, Unilever Research Laboratory Colworth Welwyn, Colworth House, Sharnbrook, Bedford MK44 1LQ

Accepted for publication 23 January 1976

SUMMARY

The importance of various unsaturated fatty acid triglycerides to the repair of faulty skin barrier function was studied in essential fatty acid-deficient rats. Following cutaneous application of the pure triglycerides for up to 5 days, the hitherto high rate of transepidermal water loss, characteristic of essential fatty acid deficiency in rats, was reduced by the triglycerides of linoleic and γ-linolenic acids. Incorporation of the applied fatty acids into the lecithin of the epidermis accompanied these changes in water loss, indicating that cutaneously applied triglycerides may be metabolized by the skin and incorporated into complex lipids. Other fatty acid triglycerides, including α-linolenic, dihomo-γ-linolenic, arachidonic and ω-7-heneicosatrienoic acid, did not lower the rate of transepidermal water loss, although all were incorporated into epidermal structural lipids. The non-essential oleic acid also had no effect upon the rate of transepidermal water loss. These data suggest that of the two main essential fatty acids that occur in skin, linoleic acid and arachidonic acid, the former specifically plays an important role in regulating barrier function whereas the latter may have a separate function, such as serving as a precursor of prostaglandins.

The condition of essential fatty acid deficiency (for comprehensive reviews, see: Alfin-Slater & Aftergood, 1968; Holman, 1968) was first observed by Burr & Burr (1929) who described the scaly skin condition and impaired rate of growth of rats maintained on fat-free diets. Subsequently, Sinclair (1952) showed that essential fatty acid-deficient rat skin was abnormally permeable to water. Basnayake & Sinclair (1956) later described the development of the characteristic skin dermatosis, abnormal transepidermal water loss and distinctive fatty acid alterations in the skin during the onset of the deficiency.

It has been known since the fundamental studies by Burr and co-workers that re-introduction of essential fatty acids to the diet of deficient animals rapidly ameliorates the condition, including the specific cutaneous manifestations, but in 1936 Shepherd & Linn showed that the caudal skin scaliness

TABLE 1. Composition of triglycerides and nomenclature of principal fatty acids

(a) Notation of triglyceride fatty acids			(b) % Fatty acid composition of triglyceride								
Fatty acid trivial name	Geneva notation	ω-Notation	18:0	18:1	18:2	α-18:3	γ-18:3	20:3	20:4	21:3	Others
Oleic	Δ-9-octadecenoic acid	ω-9-C18:1	0·7	96·5	1·7						1·1
Linoleic	Δ-9,12-octadecadienoic acid	ω-6-C18:2	0·1	5·2	93·3						1·4
α-linolenic	Δ-9,12,15-octadecatrienoic acid	ω-3-C18:3			3·2	95·6					1·2
γ-linolenic	Δ-6,9,12-octadecatrienoic acid	ω-6-C18:3	0·1		0·6		98·8				0·5
Dihomo-γ-linolenic	Δ-8,11,14-eicosatrienoic acid	ω-6-C20:3	TR	TR	TR			96·2			3·8
Arachidonic	Δ-5,8,11,14-eicosatetraenoic acid	ω-6-C20:4						5·1	91·4		3·5
—	Δ-8,11,14-heneicosatrienoic acid	ω-7-C21:3					TR	TR		92·8	7·2

TR = trace <0·1%.

TABLE 2. Transepidermal water loss through essential fatty acid-deficient rat skin following cutaneous application of various fatty acid triglycerides

Fatty acid applied to skin	Transepidermal water loss (mg/cm²/h) ± standard deviation (6)				
	Pre-treatment	Day 1	Day 2	Day 3	Day 4
C18:1ω9	2·350±0·472	2·554±0·623	3·224±0·148	2·622±0·523	2·154±0·661
C18:2ω6	2·276±0·526	2·791±0·518	1·996±0·515	1·336±0·346	1·040±0·320
C18:3ω3	2·174±0·619	2·207±0·232	3·071±0·949	3·373±0·813	2·882±0·633
C18:3ω6	2·339±0·408	2·608±0·736	1·702±0·310	1·135±0·428	0·780±0·187
C20:3ω6	3·095±1·263	4·182±0·465	2·976±0·994	3·293±0·858	2·362±0·356
C20:4ω6	1·919±0·331	3·204±1·045	4·253±1·762	4·753±2·321	4·177±0·864
C21:3ω7	2·041±0·451	2·254±0·367	2·934±0·605	2·750±0·377	2·230±0·489

Each observation is the mean of six individual readings on separate animals ± the standard deviation.

6 of the deficient rat could be cured by rubbing lard (containing essential fatty acids) onto the affected areas. Furthermore, van Dorp (1974) reported a reduction of the abnormally high rate of transepidermal water loss in deficient rats by rubbing esters of linoleic and arachidonic acid onto the skin.

We have investigated the use of cutaneous application as a means of restoring the deficiency. Cutaneous application of sunflower seed oil (rich in the triglyceride form of linoleic acid) was shown to correct the abnormal serum lipids (Press, Hartop & Prottey, 1974) and skin manifestations (Prottey, Hartop & Press, 1975) of essential fatty acid-deficient man. Recently, we have found (Prottey *et al.*, 1976) that the high rate of transepidermal water loss in EFA-deficient rats may be rapidly restored to normal by rubbing sunflower seed oil onto the skin, suggesting a close relationship between linoleic acid and the permeability of the skin to water.

The present study was undertaken to evaluate the specificity of a series of pure essential and non-essential fatty acid triglycerides in restoring abnormal skin barrier function of EFA-deficient rats and to establish the possible roles in the epidermis of essential fatty acids.

MATERIALS AND METHODS

Reagents

All chemicals and solvents used were of analytical grade of purity. Hexane was redistilled over potassium permanganate to obtain a constant boiling fraction (68 ± 1°C).

Preparation of triglycerides

As methyl esters of fatty acids are the final derivatives in many chemical procedures for preparation of pure fatty acids, these are often chosen for evaluation of biological activity by the oral route, but we found they were extremely irritant when applied to rat skin at a concentration of $500\ \mu g\ cm^{-2}$, probably due to a combination of methanol toxicity (Holman, 1968) and an irritant effect of fatty acids on skin (Kellum, 1968). In the light of these considerations, triglycerides were prepared to yield products which were biologically active (Thomasson & Gottenbos, 1962) yet non-irritant to rat skin.

Triglycerides were prepared from the following pure fatty acids: oleic acid (Sigma, London), linoleic acid, γ-linolenic acid, α-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid and ω-7-heneicosatrienoic acid (all kindly supplied by Prof. D.A. van Dorp, Unilever Research Laboratory, Vlaardingen, Netherlands). Further details of nomenclature of these fatty acids are given in Table 1a.

Fatty acid acyl chlorides were prepared by adding 3 ml oxalyl chloride (BDH Poole, England) dropwise to 3 g of the respective acid in 20 ml dry ether. The ethereal solution was refluxed under nitrogen for 2 h, cooled, and excess of reagents removed firstly under a stream of dry nitrogen, then finally under high vacuum for 2 h. The acid chloride was dissolved in 3 ml dry chloroform and added slowly with cooling to 250 mg freeze-dried glycerol in 6 ml dry pyridine-chloroform 1:1 (v/v). The solution was then sealed under nitrogen and left at room temperature for 6 days with occasional shaking. Most of the solvent was removed by rotary evaporation without heating, the residue dissolved in hexane and washed twice with ice-cold 0·5 N hydrochloric acid and finally with distilled water until neutral. The hexane solution was dried over anhydrous sodium sulphate.

The triglycerides were purified on 20 × 2 cm columns of silicic acid (Mallinckrodt 2847, 100 mesh) activated at 100°C for 1 h before use. The triglycerides were applied in hexane and washed with 100 ml of hexane to remove most of the coloured impurities from the preparations. The triglycerides were then eluted with 100 ml 5% diethyl ether in hexane (v/v). Thin-layer chromatography and infra-red spectroscopy confirmed their purity. A small aliquot of each triglyceride was transmethylated and the fatty acid purity was measured by gas chromatography. The overall yield of about 25–30% was lower than the theoretical maximum yield because an excess of fatty acid chloride to glycerol was always used to minimize the formation of mono- and diglycerides. Table 1b shows the final purities obtained.

Animals and treatment

Groups of male littermate rats of the Colworth-Wistar Albino CD inbred strain were weaned at 3 weeks on to an essential fatty acid deficient diet as described by Prottey *et al.* (1976). Animals were taken after 12 weeks on the deficient diet. The dorsal surface of each animal was clipped to remove hair and the rate of transepidermal water loss measured. Groups of six animals were treated daily on an area of shaved dorsum (4 × 5 cm) with a 0·2 ml solution of diethyl ether containing 10 mg of the respective triglyceride. The ether contained 1% butylated hydroxytoluene (w/v) as an anti-oxidant. Transepidermal water loss was measured each day, immediately before application of the triglyceride, for 5 consecutive days.

Measurement of transepidermal water loss

Transepidermal water loss was measured using a Meeco electrolytic moisture analyser (Manufacturers Engineering and Equipment Corporation, Warrington, Pennsylvania, U.S.A.) by the method described by Thiele & Schutter (1963) with modifications to accommodate small animals (Prottey *et al.*, 1976). In these experiments a fold of flank skin of the lightly anaesthetised animal (immobilized with veterinary Nembutal, Abbott Laboratories, Kent) was held in contact with the gas flow cell using a G-cramp lightly positioned against a board.

Skin separation and epidermal lipid extraction

Following the final water loss measurements, the animals were killed, the treated areas of dorsal skin were excised, and keratatome slices were removed from the whole treated area using a Castroviejo Electrokeratome (Stortz Instrument Co., St. Louis, Missouri, U.S.A.), fitted with a 200 μm shim as described by Prottey, Hartop & Ferguson (1972). These slices comprised the whole of the epidermis, together with a small portion of the upper part of the dermis, but no sebaceous glands. The slices removed from the treated area were then minced with scissors and ground with 20 ml chloroform–methanol 2:1 (v/v) in a glass tissue grinder. Each suspension was transferred to a stoppered tube and allowed to extract for 2 days at room temperature. The extract was filtered through glass fibre paper (Whatman GF/A), and the lipid extracts were washed by the method of Folch, Lees & Sloane-Stanley (1957). The extracts which contained total epidermal lipids were taken to dryness by rotary evaporation at 30°C and resuspended in 1 ml chloroform–methanol 9:1 (v/v).

Purification of phospholipids by thin-layer chromatography

Portions of total lipid extracts were applied, under a stream of nitrogen, to a thin layer (250 μm) plate (20 × 20 cm) of silica gel H (Merck AG, Darmstadt, Germany) which had been activated at 110°C for 30 min, pre-washed by developing in diethyl ether–hexane 25:75 (v/v) and re-activated after air drying. Phospholipids were separated using the solvent mixture : chloroform–methanol–glacial acetic acid–water (170:30:20:7·2, by vol.). The band representing epidermal lecithin was identified by comparison with standard dilinoleoyl lecithin (Hormel Institute, Austin, Minnesota, U.S.A.) when visualized under a uv lamp (Camag Universal uv lamp, Muttenz, Switzerland) at 254 and 360 nm. Further isolation was unnecessary as this band was almost pure when re-chromatographed on 2-dimensional TLC using the method described by Prottey *et al.* (1972). These silica bands containing the epidermal lecithin were scraped directly into tubes and transmethylated by refluxing for 90 min with methanol–benzene–conc. sulphuric acid (150:75:7·5, by vol.). The lecithin fatty acid methyl esters were extracted, evaporated to dryness under a stream of nitrogen and dissolved in 0·2 ml hexane for analysis by gas chromatography.

Gas–liquid chromatography of methyl esters

The fatty acid methyl esters were analysed isothermally at 225°C on a Pye 'series 104' dual column gas chromatograph equipped with flame ionisation detectors and 275 × 0·6 cm o.d. columns packed with 10% FFAP on 100–120 mesh Chromosorb W (Phase Separations Ltd., England). Methyl esters of saturated fatty acids from C11:0 to C24:0, mono-unsaturated even-chain fatty acids from C14:1 to C22:1, isobranched fatty acids of C14 to C24, anteiso-branched fatty acids of C15 to C23 and polyunsaturated fatty acids; linoleic α-linolenic, γ-linolenic, ω-6-eicosadienoic, ω-9-eicosatrienoic, dihomo-γ-linolenic, arachidonic and ω-7-heneicosatrienoic were used as standards.

Automatic peak integration was by a chromatographic data processor (Digital Equipment Corporation, Maynard, Massachusetts, U.S.A.), component identification was performed after programming standard retention times and identification markers. All analysis reports gave the percentage composition of all components recovered from the columns from myristic acid (C14:0) to arachidonic acid (C20:4), except the analyses from the animals receiving ω-7-heneicosatrienoic acid which included C21:3 in the total composition.

Statistical analysis

Student's paired '*t*'-test of significance was used where statistics are quoted.

RESULTS

The triglycerides are referred to in the text by their principal fatty acid, which in every case represented more than 90% of the total fatty acids. The nomenclature used, together with the number and position of double bonds in the unsaturated fatty acids are given using both Geneva and ω-notations in Table 1a.

Changes in transepidermal water loss (TEWL) after cutaneous application of various fatty acid triglycerides

Fig. 1 illustrates the changes in transepidermal water loss of essential fatty acid-deficient rats as a result of cutaneous application of various unsaturated fatty acids as triglycerides. Each plotted point is the mean of six individual observations and the values of these means and their standard deviations

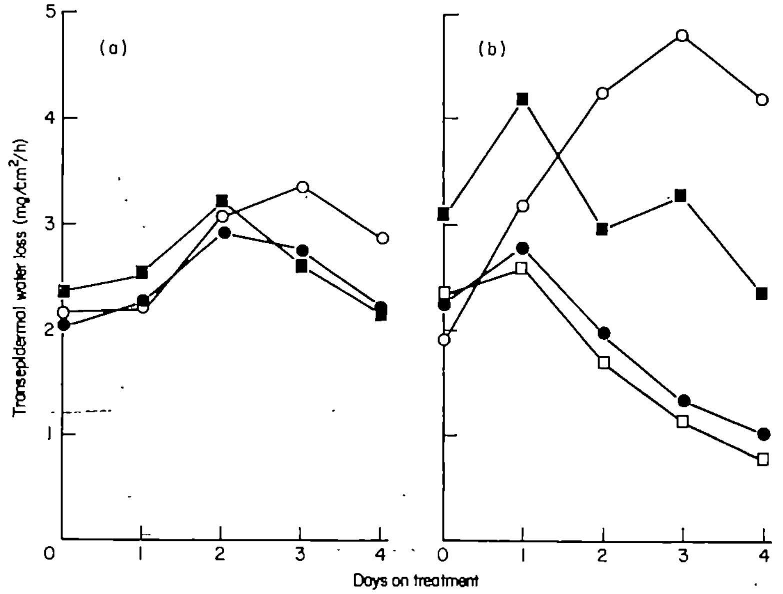


FIGURE 1. Effect of cutaneous application of pure fatty acid triglycerides on transepidermal water loss in EFA-deficient rats. (a) Non-essential fatty acids ○——○ C18:3ω3; ●——● C21:3ω7; ■——■ C18:1ω9. (b) Essential fatty acids ○——○ C20:4ω6; ●——● C18:2ω6; ■——■ C20:3ω6; □——□ C18:3ω6.

are given in Table 2. Mean pretreatment values varied between about 2 and 3 mg water loss per cm^2 per hour which is about 8–10 times that of normally fed rats of similar age. On day 1 (24 h after the first treatment) all groups showed an initial rise in the rate of TEWL, and those groups receiving α-linolenic and arachidonic acids continued to rise until after the fourth application. Oleic acid and dihomo-γ-linolenic acid did not have an overall marked effect upon transepidermal water loss apart from an initial rise in the rate of water loss. However, the ω-6-essential fatty acids, linoleic and γ-linolenic caused a consistent reduction in the rate of water loss from essential fatty acid deficient skin. Compared with the pretreatment values, γ-linolenic acid significantly reduced the TEWL after 2 days and the reduction in water loss following treatment with linoleic acid was significant after 3 days ($P = 0{\cdot}05$). This trend continued, and after 4 days both groups showed a highly significant reduction in the rate of water loss ($P < 0{\cdot}01$) compared with their pre-treatment values.

Changes in fatty acid composition of epidermal lecithin of EFA-deficient rats following cutaneous applications of triglycerides

The mean values of epidermal lecithin composition of essential fatty acid-deficient rats before application of triglycerides are given in Table 3, together with those of normal rats. Essential fatty acid deficiency of the rat was manifested by the appearance of abnormal proportions of certain fatty acids in the epidermal lecithin. Compared with normal animals there was significantly less linoleic ($P = 0{\cdot}001$) and a reduction of arachidonic acid, but significantly more palmitoleic acid ($P = 0{\cdot}01$) and the presence of ω-9-eicosatrienoic acid, which is characteristic of EFA-deficiency (Mead & Slaton, 1956).

Table 3 also shows the mean lecithin fatty acid composition of each group of deficient animals following application of the triglycerides to the skin. For each triglyceride applied it was found that the respective fatty acid of the triglyceride was incorporated into epidermal lecithin, but the mean proportional increase was different in each case. Cutaneous application of the triglyceride form of linoleic acid caused the proportion of this acid to increase in the epidermal lecithin from a value of less than 2% before treatment to about 11% afterwards. This was highly significant ($P < 0{\cdot}01$). The normal proportion of linoleic acid in rat epidermal lecithin is about 15%. Also, γ-linolenic acid (C18:3ω6) was markedly incorporated into epidermal lecithin, with several other changes in the overall fatty acid composition. The proportion of not only γ-linolenic acid itself increased to about 5·5%, but also the metabolic derivatives of this, namely, linoleic acid (the product of reduction of the Δ6 double bond) and dihomo-γ-linolenic acid (the product of chain elongation) were increased in the lecithin of the treated skin. All of these changes were highly significant ($P < 0{\cdot}01$). But the increase in linoleic acid did not reach that caused by the application of the triglyceride of this acid *per se* to the skin, and the amounts of γ-linolenate and dihomo-γ-linolenate were much greater than normally found in rat skin lecithin. Although γ-linolenic acid was found to be elongated to dihomo-γ-linolenic acid after incorporation into skin, there was no further desaturation to arachidonic acid.

Cutaneous application of the triglyceride forms of oleic (C18:1), α-linolenic (C18:3ω-3), arachidonic (C20:4ω-6), and ω-7-heneicosatrienoic acid (C21:3ω-7) resulted in the respective acids being incorporated into epidermal lecithin, in that for each of these the proportion was respectively higher after application than in untreated controls, but none of these increases was very marked.

DISCUSSION

Present results indicate that the increased permeability of the skin is restored towards normal only by linoleic (C18:2) and γ-linolenic (C18:3) acids, both of which are essential fatty acids of the ω-6-configuration. Other unsaturated fatty acids either exaggerate the deficiency or have no effect as measured by transepidermal water loss. Thus, this would exclude the possibility that the cutaneously

TABLE 3. Changes in the fatty acid composition of EFA-deficient rat epidermal lecithin following cutaneous application of various fatty acid triglycerides

Fatty acid	EFA-control	EFA-deficient							
carbon no.	NA	NA	18:1	18:2	γ-18:3	α-18:3	20:3	20:4	21:3
14:0	0·9±0·4	0·9±0·4	1·0±0·3	0·9±0·5	0·7±0·2	1·4±0·3	2·0±1·9	1·5±0·5	1·4±0·2
14:1	TR	TR	0·1±0·0	0·1±0·0	0·1±0·0	TR	TR	TR	TR
15:ant	0·4±0·3	0·1±0·0	0·1±0·0	0·2±0·1	0·2±0·1	0·1±0·0	0·1±0·1	0·1±0·1	0·1±0·0
15:0	0·8±0·4	0·7±0·2	0·8±0·1	0·6±0·3	0·6±0·1	1·0±0·1	0·8±0·8	1·0±0·2	0·9±0·1
16:iso	0·7±0·2	0·6±0·2	0·5±0·1	0·5±0·3	0·6±0·3	0·6±0·5	1·0±0·2	0·5±0·2	0·3±0·2
16:0	25·1±3·6	19·3±3·2	17·1±3·9	13·3±2·0	13·3±2·4	15·6±0·9	21·5±2·2	18·7±2·7	15·9±2·3
16:1	7·8±4·1	17·8±4·8	12·5±2·5	12·6±3·3	10·2±3·0	17·6±1·1	11·7±3·3	15·0±1·9	17·1±2·0
17:ant	0·4±0·3	0·5±0·2	0·5±0·2	0·4±0·1	0·6±0·2	0·5±0·0	0·2±0·1	0·5±0·1	0·6±0·3
17:0	0·5±0·1	0·6±0·3	0·5±0·2	0·4±0·1	0·5±0·3	0·8±0·2	0·6±0·4	0·5±0·1	0·8±0·1
17:1	1·1±0·6	1·0±0·2	0·6±0·1	0·6±0·2	0·6±0·2	0·8±0·2	0·3±0·3	0·6±0·2	0·6±0·2
18:iso	0·5±0·1	0·3±0·1	0·5±0·1	0·5±0·3	0·9±0·6	0·6±0·2	0·6±0·3	0·2±0·0	0·4±0·1
18:0	15·0±1·3	9·6±2·3	12·0±2·7	9·6±2·7	9·1±4·7	9·6±0·7	14·4±2·7	13·0±1·0	10·5±1·5
18:1	17·2±2·3	29·6±2·0	33·1±3·6	31·2±4·9	26·4±3·8	33·4±0·9	26·3±2·5	26·3±1·4	32·0±1·5
18:2	15·4±2·7	1·6±0·9	1·5±0·4	10·9±8·2	6·1±1·1	1·6±0·5	1·0±0·6	0·7±0·4	1·4±0·2
18:3ω3	—	—	—	—	—	0·7±0·5	—	—	—
18:3ω6	0·2±0·1	0·2±0·1	0·8±0·3	0·6±0·2	5·5±3·3	TR	0·4±0·1	0·2±0·1	0·3±0·2
20:iso	0·3±0·2	0·4±0·1	0·3±0·3	0·4±0·2	0·6±0·5	0·7±0·5	1·1±0·5	0·3±0·1	0·7±0·3
20:0	1·0±0·4	1·9±0·5	2·9±0·8	1·7±0·4	2·4±0·8	2·6±0·1	3·9±1·4	3·5±1·6	2·6±0.5
20:1	0·6±0·2	1·6±0·5	1·9±0·4	1·6±0·4	2·1±1·0	1·7±0·3	1·2±0·1	1·3±0·4	1·4±0·3
20:3ω9		8·6±3·4	10·9±3·7	11·6±1·4	11·9±1·4	9·4±0·8	5·3±0·8	8·1±2·4	9·0±1·4
20:3ω6	0·6±0·5	TR	0·3±0·4	TR	2·8±1·3	TR	1·0±0·6	TR	TR
20:4ω6	3·4±1·4	1·8±0·5	1·7±0·7	2·1±0·6	1·8±0·3	1·5±0·2	0·9±0·6	2·8±2·0	1·6±0·7
21:3ω7									1·5±1·0

TR = Trace less than 0·1%.
NA = no cutaneous application of triglycerides.

applied oils had merely a physical occlusive effect in lowering skin water loss for, if this were the case, then all should be as effective as linoleic or γ-linolenic acids. We have previously shown that linoleic (but not oleic acid) when applied to the skin has this ability to lower the rate of skin water loss in EFA-deficient rats (Prottey *et al.*, 1976) and in EFA-deficient man (Prottey *et al.*, 1975). Basnayake & Sinclair (1956) showed that skin permeability rapidly became normal when EFA was reintroduced to the diet. In contrast, all the fatty acids examined here, except oleic acid, have been shown to have EFA activity in promoting weight gain when fed to fat-deficient rats, using the method of Thomasson (1953). γ-Linolenic (Beerthuis *et al.*, 1968), dihomo-γ-Linolenic (Beerthuis *et al.*, 1971) and arachidonic acid (Mohrhauer & Holman, 1963; Beerthuis *et al.*, 1971) have greater EFA-activity than linoleic acid. The fatty acids α-linolenic (Mohrhauer & Holman, 1963) and ω-7-heneicosatrienoic (Beerthuis *et al.*, 1968) have been shown to exhibit less EFA activity than linoleic acid. Oleic acid was shown to have little EFA activity by this method (Alfin-Slater *et al.*, 1965). Using a subjective evaluation of the skin signs characteristic of the deficiency, Mohrhauer & Holman (1963) showed that dietary arachidonate was slightly better than linoleate at curing the deficiency, but α-linolenic acid only had limited beneficial influence upon the dermatitis. Pudelkewicz, Seuffert & Holman (1968) also found dietary linoleic acid, but not α-linolenic acid, could cure the cutaneous symptoms. It would appear that essential fatty acids are not equivalent and probably serve different functions in tissue structure and metabolism. The evidence that some can repair the impaired barrier and others cure the skin dermatosis would suggest that the two conditions are not intimately linked. Further evidence for this is provided by the appearance of a 'scaly dermatitis' during the onset of the deficiency several weeks before the change in skin permeability (Basnayake & Sinclair, 1956). These workers also showed that skin permeability and dienoic fatty acids (presumably linoleic) attained normal values rapidly when EFA was re-introduced to the diet, but the skin dermatosis only disappeared after about 5 weeks. Our own observations support these findings.

The increased skin permeability characteristic of EFA-deficiency suggests that one function of EFA is to maintain normal cutaneous membranes (Sinclair, 1958) required for barrier function. In the absence of EFA, structural phospholipids contain an altered fatty acid profile with unnatural isomers of certain unsaturated fatty acids. Table 3 shows that in EFA-deficiency there is a fall in the proportion of C18:2 in lecithin and an increase in the abnormal triene 5,8,11 eicosatrienoic acid. This acid was first shown by Mead & Slaton (1956) in EFA-deficiency and subsequently found to be derived from oleic acid (Fulco & Mead, 1959) by the same enzyme which usually forms C20:4 from C18:2 in normal animals. The switch to oleic acid as substrate only occurs when the amount of linoleic acid is limited (that is, in EFA deficiency). It is known that 5,8,11 eicosatrienoic acid is substitued for arachidonic acid in the β position of lecithin and phosphatidyl ethanolamine in EFA-deficient membranes (De Tomas, Brenner & Peluffo, 1963). Presumably the resultant phospholipids with deranged molecular configuration are unable to form functioning membranes.

A striking response to applying pure fatty acid triglycerides to the skin was the observed increase in the proportion of that fatty acid (or its own metabolic product) in the epidermal lecithin. Both linoleic and γ-linolenic acids were incorporated into EFA-deficient lecithin in appreciable amounts so as to modify the overall fatty acid composition as shown in Table 3. Although the proportion of linoleic acid was restored to almost the normal values seen in control animals, there were no measurable changes in the proportion of the 5,8,11 eicosatrienoic or archidonic acids. Peluffo, Brenner & Mercuri (1963) showed that if linoleic acid was not converted to arachidonic acid in a tissue it did not suppress the content of 5,8,11 eicosatrienoic acid in the tissue lipids. Thus this suggests that linoleic acid does not undergo desaturation and elongation to arachidonic acid in the skin. We have previously shown the rapid incorporation of linoleic acid into epidermal lecithin without alteration of the proportions of ω-9-triene and arachidonic acid in EFA-deficient rats (Prottey *et al.*, 1976) and man (Prottey *et al.*, 1975).

On the other hand, γ-linolenic acid was incorporated into skin lecithin and the results indicate it underwent not only elongation to dihomo-γ-linolenic acid but also retroconversion to linoleic acid. γ-Linolenic acid is the preferred intermediate in the conversion of linoleic acid to arachidonic acid (Struijk *et al.*, 1966) and as such is converted at high rate to C20:3ω-6 and C20:4ω-6 in many tissues. Here it was seen that the greater proportion of γ-linolenate underwent retroconversion to linoleic acid and that which underwent elongation to dihomo-γ-linolenic acid did not appear to be further converted to arachidonic acid. Presumably although the chain elongation step can occur in skin, the dehydrogenation step to a more unsaturated fatty acid may not be very active.

The present data suggest an important role of linoleic acid in the skin, from the standpoints of barrier function repair and in terms of incorporation into phospholipids. Arachidonic acid on the other hand does not seem to be involved directly in maintaining the skin barrier. In normal control rats the proportion of arachidonic acid is less than 4% of the total fatty acids in epidermal lecithin, and in EFA-deficiency the level of this acid is roughly about halved. When arachidonic acid as a triglyceride was rubbed onto the skin the amount incorporated into lecithin only marginally increased. Other workers have reported low levels of epidermal arachidonate in the rat (Ziboh & Hsia, 1971) and human skin surface (Prottey *et al.*, 1975). van Golde, Pieterson & van Deenen (1968) have observed rapid metabolism and incorporation of essential fatty acids into liver lecithin when EFA-deficient rats were fed corn oil and it may be postulated that the skin relies upon the liver as a source of endogenous arachidonic acid. The apparent readiness of essential fat-deficient skin to incorporate linoleic and γ-linolenic acid into skin lecithin and apparent reluctance to incorporate arachidonic acid as such or metabolize its immediate precursor, dihomo-γ-linolenic acid, suggest these fatty acids may play different roles in the skin. Ziboh & Hsia (1971) have demonstrated the enzymatic conversion of arachidonic acid into prostaglandin PGE_2 by homogenates of rat skin. More recently these workers showed that topical application of prostaglandin E_2 to the scaly lesions of EFA-deficient rats resulted in the clearance of the lesions, but did not significantly alter the composition of fatty acids in the skin (Ziboh & Hsia, 1972). Furthermore, PGE_2 resulted in a 70% inhibition of sterol ester biosynthesis by skin of the EFA-deficient rats. They suggested that the effects of PGE_2 in clearing the scaly dermatitis may be associated with its inhibitory effect upon abnormal sterol esterification in the skin of EFA-deficient rats. If indeed PGE_2 plays a role in the regulation of epidermal keratinization as suggested by Voorhees *et al.* (1974), it is tempting to speculate that this may be the role of arachidonic acid in skin while linoleic acid functions primarily in maintaining the integrity of the epidermal barrier. Recently we have found (unpublished observations) that cutaneous applications of PGE_1 and PGE_2 esters do not restore to normal the elevated transepidermal water loss in EFA-deficiency.

ACKNOWLEDGMENTS

Thanks are due to Miss J.E.Dalton for her technical assistance and Mr C.T.James for his synthesis of the triglycerides used in this study. We also gratefully acknowledge the discussions with Professor D.A.van Dorp at various stages of this work, and for his gift of pure lipid samples.

REFERENCES

ALFIN-SLATER, R.B., MORRIS, R.S., HANSEN, H. & PROCTOR, J.F. (1965) Effects of non-essential fatty acids on essential fatty acid deficiency. *Journal of Nutrition*, 87, 168.

ALFIN-SLATER, R.B. & AFTERGOOD, L. (1968) Essential fatty acids re-investigated. *Physiological Reviews*, 48, 758.

BASNAYAKE, V. & SINCLAIR, H.M. (1956) The effect of deficiency of essential fatty acids upon the skin. In: *Biochemical Problems of Lipids* (Ed. by G.Popjak and E.Le Breton), p. 476. Butterworths, London.

BEERTHUIS, R.K., NUGTEREN, D.H., PABON, H.J.J. & VAN DORP, D.A. (1968) Biologically active prostaglandins from some new odd-numbered essential fatty acids. *Recueil De Travaux Chimiques Des Pays-Bas*, 87, 461.

Beerthuis, R.K., Nugteren, D.H., Pabon, H.J.J., Steenhoak, A. & van Dorp, D.A. (1971) Synthesis of a series of polyunsaturated fatty acids. Their potencies as essential fatty acids and as precursors of prostaglandins. *Recueil De Travaux Chimiques Des Pays-Bas*, **90**, 943.

Burr, G.O. & Burr, M.M. (1929) New deficiency disease produced by rigid exclusion of fat from diet. *Journal of Biological Chemistry*, **82**, 345.

De Tomas, M.E., Brenner, R.R. & Peluffo, R.O. (1963) Position of eicosatrienoic acid in phosphatidylcholine and phosphatidylethanolamine from rats deficient in essential fatty acids. *Biochimica et Biophysica Acta*, **70**, 472.

Folch, J., Lees, M. & Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497.

Fulco, A.J. & Mead, J.F. (1959) Metabolism of essential fatty acids. VIII. Origin of 5,8,11, eicosatrienoic acid in the fat deficient rat. *Journal of Biological Chemistry*, **234**, 1411.

Holman, R.T. (1968) Essential fatty acid deficiency. In: *Progress in the Chemistry of Fats and Other Lipids* (Ed. by R.T.Holman) Vol. IX, pt. 2. Pergamon Press, Oxford.

Kellum, R.E. (1968) Acne vulgaris. Studies in pathogenesis: relative irritancy of free fatty acids from C_2 to C_{16}. *Archives of Dermatology*, **97**, 722.

Mead, J.F. & Slaton, W.H. (1956) Metabolism of essential fatty acids. III. Isolation of 5,8,11-eicosatrienoic acid from fat deficient rats. *Journal of Biological Chemistry*, **219**, 705.

Mohrhauer, H. & Holman, R.T. (1963) The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *Journal of Lipid Research*, **4**, 151.

Peluffo, R.O., Brenner, R.R. & Mercuri, O. (1963) Action of linoleic and arachidonic acids upon the eicosatrienoic acid level in rat heart and liver. *Journal of Nutrition*, **81**, 110.

Press, M., Hartop, P.J. & Prottey, C. (1974) Correction of essential fatty acid-deficiency in man by the cutaneous application of sunflower seed oil. *Lancet*, **i**, 597.

Prottey, C., Hartop, P.J. & Ferguson, T.F.M. (1972) Lipid synthesis in rat skin. *British Journal of Dermatology* **87**, 586.

Prottey, C., Hartop, P.J., Black, J.G. & McCormack, J.I. (1976) The repair of impaired epidermal barrier function in rats by the cutaneous application of linoleic acid. *British Journal of Dermatology*, **94**, 13.

Prottey, C., Hartop, P.J. & Press, M. (1975) Correction of the cutaneous manifestations of essential fatty acid deficiency in man by application of sunflower seed oil to the skin. *Journal of Investigative Dermatology*, **64**, 228.

Pudelkewicz, C., Seufert, J. & Holman, R.T. (1968) Requirements of the female rat for linoleic acid and linolenic acids. *Journal of Nutrition*, **94**, 138.

Shepherd, M.I., & Linn, D.R. (1936) Evaluation of vitamin F. *Drug and Cosmetic Industry*, **38**, 629.

Sinclair. H.M. (1952) Essential fatty acids and their relation to pyridoxine. *Biochemical Journal*, **51**, XVI.

Sinclair, H.M. (1958) Effects of deficiency of essential fatty acids in lower animals. In: *Essential Fatty Acids* (Ed. by H.M.Sinclair), p. 249. Academic Press, New York & London.

Struijk, C.B., Beerthuis, R.K., Pabon, H.J.J. & van Dorp, D.A. (1966) Specificity in the enzymic conversion of polyunsaturated fatty acids into prostaglandins. *Recueil De Travaux Chimiques Des Pays-Bas*, **85**, 1233.

Thiele, F.A.J. & Schutter, K. (1963) Moisture meters for measuring the water balance of the human skin. *Proceedings of the Scientific Section of the Toilet Goods Association*, **40**, 20.

Thomasson, H.J. (1953) Biological standardisation of essential fatty acids (a new method). *International Review of Vitamin Research*, **XXV**, 62.

Thomasson, H.J. & Gottenbos, J.J. (1962) Bioassay of essential acids. Comparison of effect of triglycerides, methyl and ethyl esters. *Proceedings of the Society for Experimental Biology and Medicine*, **111**, 261.

van Dorp, D.A. (1974) Essential fatty acids and prostaglandins. In: *XXIVth International Congress of Pure and Applied Chemistry*, Vol. 2, p. 117. Butterworths, London.

van Golde, L.M.G., Pieterson, W.A. & van Deenen, L.L.M. (1968) Alterations in the molecular species of rat liver lecithin by corn oil feeding to essential fatty acid deficient rats as a function of time. *Biochimica et Biophysica Acta*, **152**, 84.

Voorhees, J.J., Duell, E.A., Stawiski, M. & Harrell, E.R. (1974) Cyclic nucleotide metabolism in normal and proliferating epidermis. In: *Advances in Cyclic Nucleotide Research* (Ed. by P.Greengard and G.A.Robison), Vol. 4, p. 117. Raven Press, New York.

Ziboh, V.A. & Hsia, S.L. (1971) Prostaglandin E_2: biosynthesis and effects on glucose on lipid metabolism in rat skin. *Archives of Biochemistry and Biophysics*, **146**, 100.

Ziboh, V.A. & Hsia, S.L. (1972) Effects of prostaglandin E_2 on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. *Journal of Lipid Research*, **13**, 458.

British Journal of Dermatology (1976) **95**, 265.

The diffusion of nandrolone through hydrated human cadaver skin

M.I.FOREMAN AND I.KELLY

Scientific Development Group, Organon Laboratories Ltd., Newhouse, Lanarkshire ML1 5SH, Scotland

Accepted for publication 28 January 1976

SUMMARY

Effective diffusion constants have been determined for the permeation of nandrolone through whole and stripped human abdominal cadaver skin *in vitro*. From the values obtained, the diffusion constant for nandrolone in the stratum corneum has been inferred. The experimental approach used yields reproducible results for skin samples taken from a single individual. There is also a surprising degree of uniformity in the values obtained for skin from different individuals.

The study of diffusion in skin is usually restricted to the measurement of the permeability coefficient (P) under conditions of steady state flow. In the present work a more fundamental parameter, the diffusion constant (D) has been determined for samples of human abdominal cadaver skin by a refinement of the 'lag-time' method (Daynes, 1920; Barrer, 1951). In conventional applications of this method, a plot of Q_t, the total amount of material which crosses the membrane in the time interval 0 to t, is obtained as a function of t. The plot has the general form of Fig. 1, consisting of a curved region, which corresponds to transient flow conditions within the membrane, and finally a linear region when steady state conditions have been attained. Normally the linear region of the curve is extrapolated back to the t-axis to obtain a measure of the 'lag-time' for the membrane (τ_L), which is related to the diffusion constant by the expression:

$$\tau_L = \frac{l^2}{6D} \tag{1}$$

l being the membrane thickness. In the above treatment therefore, all data on the curved region of the plot are effectively discarded. Further, in order to obtain a satisfactorily accurate extrapolation, measurements must be taken under steady state conditions for as long a time as possible. For studies involving cadaver skin, where experiments of protracted duration are impracticable for obvious reasons, it is clearly an advantage to utilize all of the data obtained, particularly that on the curved region of the plot. In the analysis used here, all of the data recorded were fitted by an iterative least-squares computer programme (Wolberg, 1967) to the full equation for the curve:

$$\frac{Q_t}{AlKC_0} = \frac{Dt}{l^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot \exp\left(-Dn^2\pi^2\, t/l^2\right) \tag{2}$$

where A is the area of the skin surface exposed to the solvent, K the membrane–solvent partition coefficient, and C_0 the concentration of diffusing material just within the membrane surface. (In computing the least-squares fit, the infinite series was truncated when a term was encountered which added less than 0·1% to the accumulated total.) In this way, effective diffusion constants and partition coefficients have been determined for stripped and whole human abdominal cadaver skin. By suitable manipulation of these data, it has also proved possible to obtain a value for the diffusion constant in the stratum corneum.

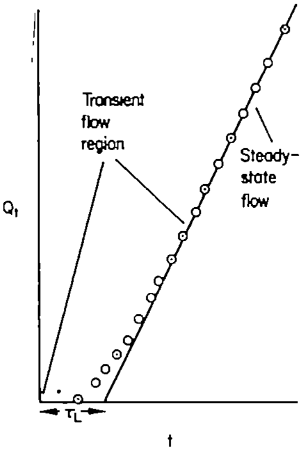


FIGURE 1. Ideal plot of Q_t versus t under the conditions of the 'lag-time' experiment.

MATERIALS

Samples of human abdominal cadaver skin were obtained at autopsy. On receipt, all subcutaneous fat was removed. Samples were stored under refrigeration (−4°C). The thickness of the samples was measured by micrometer gauge immediately before use, after equilibrating to room temperature. Stripped skin was prepared as required by repeatedly applying Scotch Tape until the glistening layer was reached. Generally 14 strippings were required. Nandrolone-4-^{14}C of specific activity 3·14 mCi/mg was used as supplied by Fluorochem Ltd.

Bray's scintillation liquid was prepared from 64 g naphthalene, 4 g 2,5-diphenyloxazole and 200 mg 2,4-di-2-(5-phenyloxazolyl) benzene in 100 ml methanol to which 20 ml ethylene glycol had subsequently been added, the volume being made up to 1 litre with 1,4-dioxan.

METHODS

Diffusion studies were carried out in the following manner: Skin samples were mounted in the glass diffusion cells illustrated in Fig. 2, with the dermal surface in contact with the sink solvent, the epidermal surface in contact with the reservoir solution. A solution of nandrolone-4-^{14}C was pumped continuously through one chamber of the cell from a 100 ml capacity reservoir. Simultaneously, solvent from a 100 ml capacity sink was pumped around the second chamber of the cell. The flow

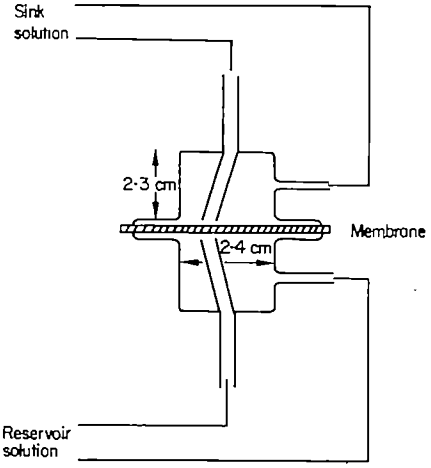


FIGURE 2. Schematic diagram of glass diffusion cell.

of both solvents was maintained by a Watson-Marlow HR flow inducer. Although loss of material into the plastic tubing included in the flow circuits was found to be negligible for this particular system, the use of such tubing was reduced to a minimum, and appropriate checks were carried out. At suitable time intervals the sink was removed from the apparatus and replaced by fresh solvent, such that the concentration of diffusing material in the sink was never greater than 1% of that in the reservoir, and may therefore be considered to have been zero throughout. The concentration of diffusant within the reservoir was also monitored during the experiment, and restored to the original value whenever necessary. The reservoir concentration was therefore effectively constant throughout the experiment, and at no time changed by more than 5%. Under these conditions, Equation 2 applies. The amount of radioactive material within the sink and reservoir at any given time was estimated by liquid scintillation counting using a Unilux II counter.

Preliminary experiments using methods described previously (Foreman & Clanachan, 1975) established that the radiochemical purity of the nandrolone was not significantly degraded on passage through cadaver skin under the present conditions.

RESULTS AND DISCUSSION

It must be recognized at the outset, that by the very nature of the experimental conditions, the present results relate to skin which is saturated with solvent, in this case 25% ethanol in water. At best therefore, the measurements relate to the *in vivo* situation of rigorous occlusion.

Nevertheless, the results obtained from this study seem very promising. Initially, two separate measurements were carried out on samples of stripped abdominal cadaver skin taken from a single donor. The results obtained (Runs 1 and 2, Table 1) show an acceptable level of reproducibility. Subsequently, measurements were carried out on a further skin sample from a different source (Run 3, Table 1), from which it may be seen that the agreement between the values obtained for the two different skin samples is surprisingly good. In all cases, the fit between the observed and calculated Q_t versus t data based on Equation 2 is excellent, including the data obtained under transient flow conditions. This is illustrated for a typical run in Fig. 3. It is also evident from this figure that the conventional treatment for data of this kind would effectively waste some 25% of the measured points.

The mean value for the diffusion constants quoted in Table 1 for stripped skin represents an effec-

TABLE I. Diffusion constants and partition coefficients for nandrolone diffusion in stripped human abdominal cadaver skin at 22 ± 2°C

Run	Skin thickness (cm)	Partition coefficient	Diffusion constant ($cm^2\ s^{-1}$)
1	0·266	0·65	$2·8 \times 10^{-7}$
2	0·276	0·57	$3·4 \times 10^{-7}$
3	0·259	0·50	$2·4 \times 10^{-7}$

Mean values: Partition coefficient, 0·57 ± 0·05; Diffusion constant, $2·9 \pm 0·3 \times 10^{-7}$ $cm^2\ sec^{-1}$.

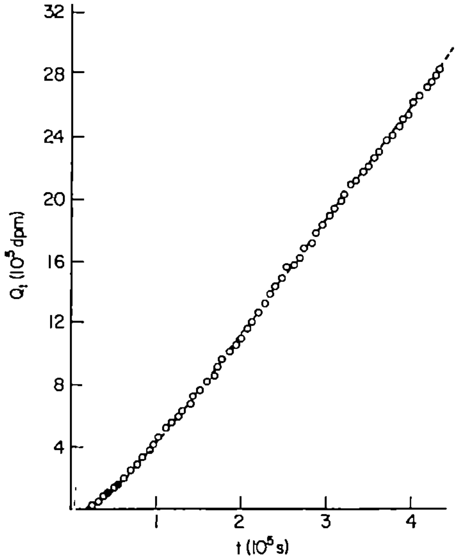


FIGURE 3. Plot of observed Q_t versus t data for stripped skin Run 3. Dashed line is calculated for the parameters quoted in Table 1.

tive diffusion constant for the combined viable epidermis and dermis. It is assumed in what follows that the diffusion constant for the viable epidermis is not greatly different from that for the dermis, and that the above figure is representative of the combined viable epidermal and dermal layers. Of greater interest is the diffusion constant for nandrolone in the stratum corneum. An estimate of this quantity was obtained in the following way. A membrane may be considered to offer a resistance R to diffusion, where R = 1/D. For a laminated membrane such as skin, provided that the partition coefficient for the diffusing molecule is unity between the various layers, then the resistance of the whole membrane is the sum of the resistances of the separate layers;

$$R = \Sigma R_i \tag{3}$$

For a sample of whole skin therefore,

$$R_{ws} = R_{sc} + R_{ed} \tag{4}$$

where R_{ws} is the resistance of whole skin, R_{sc} that of the stratum corneum and R_{ed} that of the viable epidermal–dermal layer. Consequently, if the effective diffusion constant is measured for whole skin, it should be possible to estimate D_{sc} using the measured value of D_{ed} if the thickness of the stratum corneum is known. Unfortunately, this is not a parameter which is easily measured. It has therefore been assumed in what follows that the thickness of the stratum corneum of human abdominal skin is 0·001 cm (Scheuplein, 1972).

The effective diffusion constant for nandrolone in various samples of whole human cadaver skin was next measured in the manner described above, and R_{ws} calculated for each sample. R_{ed} was estimated using the mean value for D_{ed} from Table 1. Values of D_{sc} obtained in this way are quoted in Table 2. In one instance, measurements were carried out under conditions of transient flow only. The data obtained are shown in Fig. 4 and the derived parameters quoted in Table 2 (Run 4). From the figure, it can be seen that the calculated and observed curves agree very well, and the estimated dif-

TABLE 2. Diffusion constants and partition coefficients for nandrolone diffusion in whole human abdominal cadaver skin at 22 ± 2°C

Run*	Skin thickness (cm)	D_{ws} ($cm^2\ s^{-1}$)	Partition coefficient	R_{ws} ($cm^{-1}\ s$)	R_{ed} ($cm^{-1}\ s$)	R_{sc} ($cm^{-1}\ s$)	D_{sc} ($cm^2\ s^{-1}$)
1	0·212	$4{\cdot}9\times10^{-8}$	1·9	$4{\cdot}3\times10^{6}$	$7{\cdot}1\times10^{5}$	$3{\cdot}6\times10^{6}$	$2{\cdot}8\times10^{-10}$
2	0·210	$5{\cdot}3\times10^{-8}$	2·1	$4{\cdot}0\times10^{6}$	$7{\cdot}3\times10^{5}$	$3{\cdot}3\times10^{6}$	$3{\cdot}0\times10^{-10}$
3	0·072	$1{\cdot}6\times10^{-8}$	3·7	$4{\cdot}5\times10^{6}$	$2{\cdot}5\times10^{5}$	$4{\cdot}3\times10^{6}$	$2{\cdot}3\times10^{-10}$
4	0·102	$3{\cdot}7\times10^{-8}$	1·9	$2{\cdot}8\times10^{6}$	$3{\cdot}5\times10^{5}$	$2{\cdot}5\times10^{6}$	$4{\cdot}0\times10^{-10}$

* Runs 1 and 2 were carried out on skin from a single donor.

Mean diffusion constant for the stratum corneum = $3{\cdot}1 \pm 0{\cdot}4\times10^{-10}$ cm² s⁻¹.

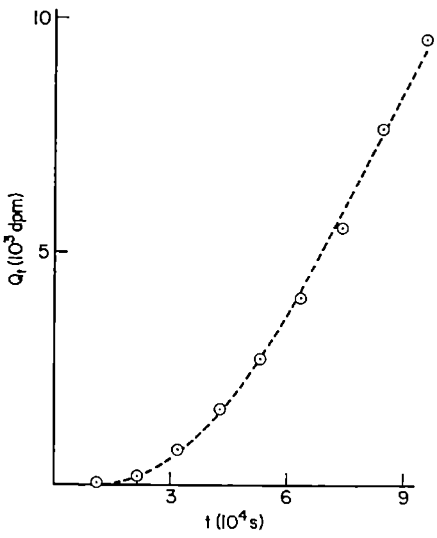


FIGURE 4. Data obtained for whole skin Run 4 under conditions of transient flow. Dashed line is calculated for the parameters quoted in Table 2.

fusion constant and partition coefficient are in good accord with the values determined from more extended studies. That the observed data can be fitted to Equation 2 so well suggests not only that it is possible to derive the diffusion parameters from a study of transient flow behaviour alone, but also that shunt diffusion (Scheuplein, 1972) is probably unimportant in this particular case, since penetration via shunt pathways should be most apparent under conditions of transient flow, and would cause the observed data to deviate appreciably from Equation 2 in this region of the curve. This appears, however, not to be the case.

Two other points of interest become evident from a consideration of Table 2. Firstly, the effective diffusion constant for whole skin decreases with decreasing skin thickness. This would seem to justify the assumption that abdominal stratum corneum is of more or less constant thickness in samples of normal skin. The stratum corneum would then comprise a greater proportion of the total skin in the thinner samples, and thereby reduce the measured diffusion constant. The second point worthy of note is the very good agreement between the derived values of D_{sc} obtained for samples of skin from different individuals. This level of agreement tends to support the validity of the approach used here, and, taken together with the results obtained for the stripped skin samples, suggests that the diffusion constants for a given molecule in the various skin layers do not vary greatly from individual to individual, at least for physiologically normal skin.

It was indicated in the discussion earlier that Equation 3 is only valid in the present context if the partition coefficient for nandrolone between the stratum corneum and the viable epidermis is unity. It is possible to obtain an indication of the validity of this assumption in the following way. From Table 2, the mean coefficient for partition of nandrolone between the solvent and the upper surface of the stratum corneum is 2·8, that for partition between the solvent and the upper surface of the viable epidermis is 0·57. Consequently, the partition coefficient between the stratum corneum and the viable epidermis is of the order of $2{\cdot}8/0{\cdot}57 = 5$. However, strictly speaking, the required coefficient is that for the *lower* face of the stratum corneum. If, as seems reasonable, the stratum corneum becomes less lipophilic towards the lower surface, the true value of this partition coefficient will therefore be less than 5, and the approximation that it is equal to unity seems a valid one.

In conclusion, an attempt has been made in this work to place the study of molecular diffusion in skin on a somewhat more rigorous physicochemical basis than has generally been the case hitherto. The value of extracting diffusion parameters which have the highest possible degree of thermodynamic validity under the given circumstances cannot be too highly emphasized, if a proper understanding of the nature of skin as a barrier membrane is to be fully understood.

REFERENCES

BARRER, R.M. (1951) *Diffusion in and through solids*, 1st edn, p. 18. Cambridge University Press, Cambridge.

DAYNES, H. (1920) The process of diffusion through a rubber membrane. *Proceedings of the Royal Society*, **A97**, 286.

FOREMAN, M.I. & CLANACHAN, I. (1975) The percutaneous penetration of nandrolone decanoate. *British Journal of Dermatology*, **93**, 47.

SCHEUPLEIN, R.J. (1972) Properties of the skin as a membrane. In: *Advances in Biology of the Skin, Pharmacology and the Skin* (Ed. by W. Montagna, R.B. Stoughton and E.J. Van Scott), Chap. 10, p. 125. Appleton-Century-Crofts, New York.

WOLBERG, J.R. (1967) *Prediction Analysis*, 1st edn, p. 27. Van Nostrand, New York.

British Journal of Dermatology (1976) **95**, 271.

Lysosomal hydrolases of the epidermis

6. CHANGES IN DISEASE

P.D.MIER AND JOSÉ J.M.A.VAN DEN HURK

Department of Dermatology, University of Nijmegen, The Netherlands

Accepted for publication 27 January 1976

SUMMARY

The activities of 14 acid hydrolases have been measured in normal and diseased human epidermis. Our findings were as follows: (1) Increased hydrolase activity was common in lesions; the clinically uninvolved skin of patients, however, invariably showed normal values. (2) The *pattern* of change was independent of the nature of the disease, the arylsulphatases and β-glucosidase usually showing the greatest elevations. (3) The *magnitude* of the changes differed from disease to disease, being most marked in the lesions of psoriasis. (4) Ichthyosiform erythroderma was exceptional in showing elevated levels of the galactosidases and cathepsins B_1 and D. We suggest that (with the exception of ichthyosiform erythroderma) the abnormalities which we have observed may be related to the proliferative rate of the epidermis.

Although a few reports have indicated that elevated hydrolase activity may be a feature of certain dermatoses (reviewed by Lazarus, Hatcher & Levine, 1975), little quantitative biochemical information is available. We have therefore surveyed the levels of 14 acid hydrolases in lesions and in clinically uninvolved epidermis from patients with various epidermal disorders; our findings are presented below. The values for psoriasis have been reported in a preliminary communication (Mier & van den Hurk, 1976).

MATERIALS AND METHODS

The majority of patients included in the investigation were attending this Department at the time of biopsy; in some cases patients were recalled by letter. Control specimens were obtained from healthy paid volunteers (aged 16–43 years) who had no history of skin disease.

Lesions selected for biopsy were, as far as possible, typical of the disease represented, and had been untreated for at least 2 weeks. All specimens were taken from the trunk, thigh or upper arm. Epidermis (average about 40 mg fresh weight) was cut using a Castroviejo keratotome set for a depth of 0·2 mm, after freezing the skin surface with ethyl chloride spray.

Analyses were generally performed in batches of 4–6 specimens, at least one control specimen being included in each batch. Immediately prior to analysis specimens were weighed and homogenized in 2 ml bovine serum albumin solution (1 mg/ml) using an all-glass homogenizer at 0°C, and centri-

TABLE 1. Acid hydrolase activities in some disorders of the epidermis

Specimen	α-glu	β-glu	α-gal	β-gal	α-man	β-NAG	β-gln	AP	sf-A	sf-B	cat B$_1$	cat C	cat D	aram
Controls (46)														
Highest	25	4·8	18	91	64	450	160	1900	1·5	1·1	11	130	3·5	610
Mean	11	1·7	10	42	28	130	50	880	0·51	0·46	4·9	51	1·9	300
Lowest	2·9	0·14	5·0	8·9	11	32	5·1	280	0·06	0·05	1·8	19	0·72	85
Lesions														
Atopic prurigo (4)	33	8·6	17	73	57	260	180	3000	4·0	3·0	4·9	97	1·1	740
Darier's disease (3)	20	4·7	15	66	36	240	76	2400	1·2	1·1	4·7	43	2·3	470
Eczema (4)	19	4·3	10	45	28	140	88	1300	1·4	1·2	3·9	96	2·5	560
Erythema multiforme (1)	18	3·1	9·6	48	18	130	69	460	0·49	0·45	4·9	59	1·4	160
Ichthyosiform erythroderma (2)	13	2·4	27	100	23	220	48	1500	2·0	1·6	13	55	9·1	520
Ichthyosis vulgaris (6)	16	3·9	13	60	33	150	53	1800	1·5	1·0	4·6	40	2·7	440
Lichen planus (5)	17	6·3	15	59	24	140	68	1600	1·5	1·2	4·7	71	2·5	690
Psoriasis (8)	21	14	16	58	37	170	83	2200	16	9·4	6·3	120	6·3	910
Uninvolved epidermis														
Atopic (3)	15	2·9	11	61	23	140	63	840	0·59	0·48	3·6	29	1·9	430
Darier's disease (2)	15	2·2	13	77	21	200	42	1200	0·43	0·40	5·7	29	1·3	400
Ichthyosiform erythroderma (1)	19	3·2	13	54	23	130	56	850	0·56	0·77	3·9	52	1·1	440
Ichthyosis vulgaris (4)	9·5	1·3	11	36	25	170	19	1000	0·37	0·24	4·8	52	1·6	290
Lichen planus (4)	16	1·9	9·5	52	22	160	54	1000	0·36	0·36	4·2	56	3·1	370
Palmoplantar keratoderma (1)	8·7	1·4	8·7	39	19	100	33	860	0·45	0·37	3·4	53	2·2	320
Psoriasis (7)	11	1·5	11	39	26	100	50	900	0·42	0·48	5·0	50	1·9	300

Values are in nmol/min/g wet weight. Abbreviations are: glu = glucosidase; gal = galactosidase; man = mannosidase; NAG = N-acetylglucosaminidase; gln = glucuronidase; AP = acid phosphatase; sf = sulphatase; cat = cathepsin; aram = arylamidase. EC numbers are as listed previously (Mier & van den Hurk, 1975 a–c). Figures in parenthesis indicate the number of specimens assayed; in the case of the controls this includes 36 values reported in a preliminary publication (Mier & van den Hurk, 1976).

fuged. Appropriate dilutions were prepared in bovine serum albumin solution, and the enzyme activities determined in duplicate using techniques already described (Mier & van den Hurk, 1975a–c).

RESULTS

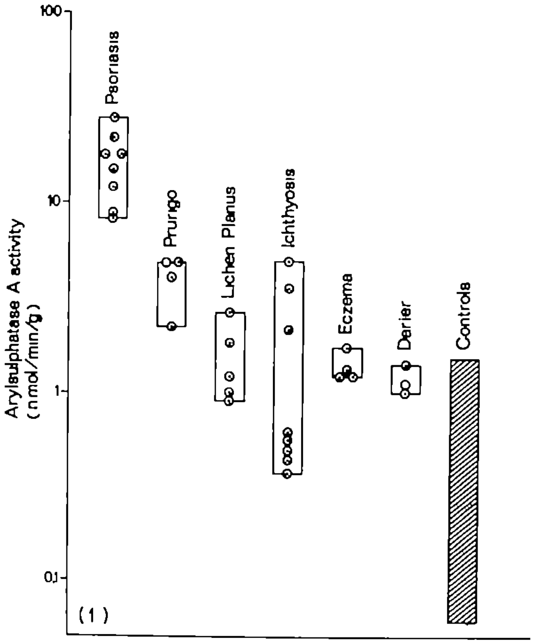
The levels of hydrolase activity in normal, diseased and clinically uninvolved epidermis are summarized in Table 1. It is seen that the pattern of change is similar in most kinds of lesion. The arylsulphatases and β-glucosidase display by far the greatest elevations; arylamidase and acid phosphatase show significant but less marked increases, and the remaining enzymes are relatively unaffected. The only clear exception to this pattern is ichthyosiform erythroderma, the lesions of both patients showing elevated levels of the galactosidases and cathepsins B_1 and D.

The enzyme levels in the individual specimens are illustrated in the cases of arylsulphatase A and β-glucosidase (Figs 1 & 2). It is clear that the magnitude of the change varies considerably from disease to disease, although being fairly constant for any one condition.

Statistical analysis revealed no significant correlation of acid hydrolase activity with the sex or age of the donors or with the site of biopsy.

DISCUSSION

Workers in several different fields have pointed out that a relationship exists between the mitotic rate of a particular tissue and its level of hydrolase activity. Elevated hydrolases have been reported, for example, in neonatal liver and kidney (Franklin, 1962), in regenerating liver (Roy, 1958), in



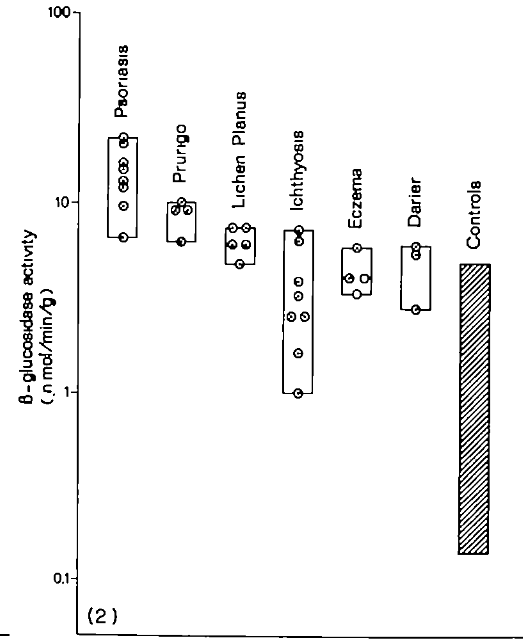


FIGURE 1. The levels of activity of arylsulphatase A (nmol/min/g fresh weight) in the individual lesions. There is a significant elevation at the level $P < 0.01$ for all diseases except ichthyosis and Darier's disease (Wilcoxon ranking test).

FIGURE 2. The levels of activity of β-glucosidase (nmol/min/g fresh weight). There is a significant elevation at the level $P < 0.01$ for all diseases except Darier's disease (Wilcoxon ranking test).

many types of solid tumour (reviewed by Poole, 1973) and in cultured cells following viral transformation (Bosmann, 1972, 1974). Were such a relationship to pertain also to epidermis, it would provide a simple interpretation of the data which we have presented.

Although mitotic parameters are available for few dermatoses other than psoriasis, our figures for the individual diseases are by no means incompatible with this view. It is also consistent with the increased hydrolase activity reported in epidermal tumours (Shamberger & Rudolph, 1967) and with our recent observation that, following a single dose of ultraviolet radiation, elevated hydrolase activity coincides with the period of 'post-injury cell renewal' (manuscript in preparation). Finally, it seems relevant that the two enzymes which display a particularly clear correlation (Figs 1 & 2) do indeed occur chiefly in the proliferative region of the epidermis; acid phosphatase, which shows rather irregular increases is located mainly in the keratinizing zone (Mier *et al.*, 1976).

Thus it seems likely that a relationship between the mitotic rate and the levels of activity of certain acid hydrolases is an element in the normal mechanism of growth control, and that increased hydrolase activity is an incidental, rather than a 'causative' phenomenon in diseases characterized by increased proliferation. Nevertheless, the question of how these enzymes influence mitosis (or *vice versa*) is of great importance. Several authors have proposed that hydrolases may modify the 'surface architecture' of the plasma membrane, thereby releasing the cell from contact inhibition (see discussion by Elligsen, Thompson & Frey, 1975). We have already drawn attention to the converse possibility, namely that an increased level of membrane glycoconjugates could result in the induction of hydrolase activity (Mier & van den Hurk, 1976). Further speculation must await a better knowledge of these macromolecules and their role in growth control.

ACKNOWLEDGMENTS

The authors wish to thank Professor J.W.H.Mali and the medical staff of this Department for their help in providing the clinical material used in this work.

REFERENCES

Bosmann, H.B. (1972) Elevated glycosidases and proteolytic enzymes in cells transformed by RNA tumour virus. *Biochimica et Biophysica Acta*, **264**, 339.

Bosmann, H.B. (1974) Surface biochemical changes accompanying primary infection with Rous sarcoma virus. 2. Proteolytic and glycosidase activity and sublethal autolysis. *Experimental Cell Research*, **83**, 25.

Elligsen, J.D., Thompson, J.E. & Frey, H.E. (1975) An evaluation of lysosomal enzyme leakage as a factor influencing the behaviour of transformed cells. *Experimental Cell Research*, **92**, 87.

Franklin, T.J. (1962) The influence of age on the activities of some acid hydrolases in the rat liver and kidney. *Biochemical Journal*, **82**, 118.

Lazarus, G.S., Hatcher, V.B. & Levine, N. (1975) Lysosomes and the skin. *Journal of Investigative Dermatology*, **65**, 259.

Mier, P.D. & van den Hurk, J.J.M.A. (1975a) Lysosomal hydrolases of the epidermis. 1. Glycosidases. *British Journal of Dermatology*, **93**, 1. *Idem* (1975b) 2. Ester hydrolases, **93**, 391. *Idem* (1975c) 3. Peptide hydrolases, **93**, 509.

Mier, P.D., Cotton, D.W.K., van den Hurk, J.J.M.A. & Jonckheervanneste, M.M.H. (1976) Lysosomal hydrolases of the epidermis. 5. Variation with depth in the cow snout. *British Journal of Dermatology*, in press.

Mier, P.D. & van den Hurk, J.J.M.A. (1976) Acid hydrolases in psoriatic epidermis. *British Journal of Dermatology*, **94**, 219.

Poole, A.R. (1973) Tumour lysosomal enzymes and invasive growth. In: *Lysosomes in Biology and Pathology* (Ed. by J.T.Dingle), Vol. 3, p. 303. North-Holland Publishing Co., Amsterdam.

Roy, A.B. (1958) Comparative studies on the liver sulphatases. *Biochemical Journal*, **68**, 519.

Shamberger, R.J. & Rudolph, G. (1967) Increase of lysosomal enzymes in skin cancers. *Nature*, **213**, 617.

British Journal of Dermatology (1976) 95, 275.

The fate of Staphylococcal exfoliatin in newborn and adult mice*

PETER FRITSCH, PETER ELIAS† AND JANOS VARGA

Department of Dermatology, Yale University, New Haven, Connecticut, and †Departments of Pathology and Dermatology, University of California, San Francisco, California, U.S.A.

Accepted for publication 9 January 1976

SUMMARY

The distribution and excretion of the staphylococcal exfoliatin was investigated following *in vivo* administration of highly purified ^{125}I-labelled exfoliatin fractions to adult and newborn mice. Adult mice excrete approximately one-third of a test dose by 3 hours as compared to a fifteenth of a test dose excreted by newborn mice. Accordingly, blood tracer radioactivity reaches a relatively higher peak and shows a slower decline in newborns than in adults. The urine of adult mice contains considerable biologically active exfoliating material. Both nephrectomized and carbon tetrachloride-poisoned adult mice injected with exfoliatin develop generalized exfoliation whereas comparable doses in untreated controls have no effect. On the other hand, subtotal hepatectomy, followed by injection of exfoliatin, does not lead to exfoliation. We conclude that renal immaturity is a critical factor responsible for the susceptibility of neonates to generalized staphylococcal scalded skin syndrome.

Generalized staphylococcal scalded skin syndrome (SSSS) is most frequently encountered in neonates and infants. This age predilection is so characteristic that, previously, neonatal age was considered a prerequisite for the diagnosis (Ritter von Rittershain, 1975; Tachau, 1934). Today, cases are recognized in childhood and, rarely, even in adults (Epstein, Flynn & Davis, 1974; Hawley & Aronson, 1973; Levine & Norden, 1972; Reid, Weston & Humbert, 1974; Rothenberg *et al.*, 1973). Yet, analysis of representative recent series indicates that no shift of the peak incidence has taken place (Elias, Fritsch & Epstein, 1976).

Experimental data utilizing the recently developed murine model (Melish & Glasgow) are consistent with this clinical experience: mice injected with live phage group 2 staphylococci are susceptible to generalized SSSS only as neonates. Moreover, an extracellular product here termed exfoliatin (ET) has been isolated from these organisms and similarly produces generalized SSSS only in neonatal mice (Arbuthnott *et al.*, 1972; Melish & Glasgow, 1972). Thereafter, they react only locally at the site of injection (Elias *et al.*, 1974; Fritsch, 1975). These findings, coupled with the discovery that human and murine skin of all ages is cleaved readily by ET *in vitro*, suggest that the age difference in responsiveness is due to extracutaneous factors.

---

* Portions of this work were presented to the Western Regional Meeting of the American Federation of Clinical Research, Carmel, California on 5 February 1976.

Reprint requests to: Dr P.Fritsch, Div. Exp. Derm., Dept. Derm. I. University of Vienna, School of Medicine, A-1090 Vienna, Alserstrasse 4, Austria.

Speculation about the factor(s) responsible for adult resistance has centred around anatomical (hair development) (Arbuthnott, Kent & Noble, 1973; Kapral & Miller, 1972) and immune mechanisms (Reid *et al.*, 1974; Elias *et al.*, 1974) or enhanced metabolism (Elias *et al.*, 1974). In this study we present data which suggest that the development of generalized SSSS is linked to the host's ability to excrete and/or metabolize the staphylococcal ET.

MATERIALS AND METHODS

Purification of exfoliatin

Crude ET was prepared by 40% and 80% ammonium sulphate precipitation of *Staphylococcus aureus* phage group 2 culture media as previously described (Elias *et al.*, 1974). The 80% precipitate was purified by isoelectric focusing in polyacrylamide gels (Awdeh, Williamson & Askonas, 1968) containing urea (2 M) and ampholytes, pH 3–01 (Bio-Rad). The active fraction, determined by injection into newborn mice, was found at pH 7·2 corresponding to a band in a Coomassie blue stained gel. ET was eluted in phosphate buffered saline (PBS), dialysed extensively against distilled water, and lyophilized. The threshold dose of ET was approximately $0·5 \times 10^{-6}$ g/g body weight in 1–2-day-old mice.

Preparation of labelled exfoliatin

ET was labelled with ^{125}I by the chloramine T method (Greenwood, Hunter & Glover, 1963) as modified by Varga *et al.* (1974). The iodinated exfoliatin (I-ET) was separated from the other components of the reaction on a $0·7 \times 30$ cm Sephadex G25 column equilibrated with PBS pH 7·4, and by subsequent dialysis against PBS. Specific radioactivity was approximately 20 Ci/mMol. Serial dilutions tested in newborn mice revealed no loss of biological potency as compared to similar concentrations of cold ET.

Experimental material

Four newborn and four young adult wild type albino mice and a newborn wild type albino rat received subcutaneous injections of I-ET doses ranging from 20·000–150·000 c/min/g body weight. Blood from the tip of the tail and urine samples were taken at regular intervals from 0 to 48 h. In another set of experiments urine was collected over a 3-h period. At the end of the experiments, the animals were sacrificed and weighed parts of various tissues as well as blood and urine samples were measured separately in an AW-1450 Gamma counter (Scientific Products).

For determination of the biological activity of ET in the urine, adult mice received subcutaneous injections of a mixture of crude ET (80% ammonium sulphate precipitate) with I-ET (1000 c/min/mg crude ET) at a dose of 1 mg crude ET/g body weight. The urine was collected over a 3-h period and its total radioactivity was measured. The urine samples were then pooled, dialysed against water, lyophilized and dissolved in PBS to give a final concentration of 1000 c/min/ml. Newborn mouse skin was incubated for 2 h at 37°C in this solution in serial dilutions and assayed for exfoliation. Controls were incubated in dilutions of the original crude ET-I-ET mixture. Exfoliation was documented as previously described (Elias *et al.*, 1974) by peeling off the superficial layers of the epidermis, by histology, and smear preparations.

For induction of SSSS in metabolically compromised adult mice, chemical depilation of the backs with thioglycollate agents was performed 24 h prior to the experiments. In previous studies (Elias *et al.*, 1974) this treatment proved to have no effect on the susceptibility of the skin to ET. Four animals were nephrectomized and three were subtotally hepatectomized (approximately 5/6) by ligation and excision of these organs under ether anaesthesia. They were given 20 mg crude ET intramuscularly into the thighs immediately after the operation. This dose is 20 times the minimum required to produce exfoliation in newborn mice on an equal weight basis. Sham-operated animals given the same ET-dose served as controls. All animals were tested for generalized SSSS after 4 h by attempts to elicit the Nikolski sign on the back skin and by histology and smear preparations (Elias *et al.*, 1974).

Carbon tetrachloride (CCl_4) was administered to pre-epilated adult mice at a final concentration of 0·01 ml/g body weight in a 1:1 dilution of CCl_4 in mineral oil. Solutions were injected subcutaneously or intraperitoneally. After 24 h, four animals, exhibiting obvious signs of toxicity, were injected with 20 mg crude ET. Two carbon tetrachloride-treated mice without ET-injection and two others which had been treated with mineral oil alone and were injected with ET served as controls. All animals were tested for generalized SSSS as above and toxic effects in liver and kidneys were ascertained by gross and microscopic pathology.

RESULTS

Blood and urine tracer levels

Adult mice. Blood tracer levels peaked 2 h after injection followed by a logarithmic decline (Fig. 1a). The half-life of the tracer in the blood was approximately 10 h. The relationship between peak blood tracer levels and the various doses injected on body weight basis was linear, the quotient being 1·18 ± 0·06.

Urine radioactivity rose sharply after approximately 60 min, reaching a peak of up to 100 × blood levels at 2 h (Fig. 1d). At 10 h, urine tracer levels returned to 2-fold the blood levels, and subsequently declined slowly. One-third of the tracer injected was recovered in urine samples collected over 3 h (Table 1).

TABLE 1. Excretion rate of tracer into urine

	Total tracer activity in urine after 3 h as % of I-ET injected*	
Newborn mouse	6·4	7·2
Adult mouse	33·0	37·2

* Single measurements.

Newborn mice. Blood tracer levels also rose quickly peaking at 2 h but the decline was much slower (Fig. 1b). Again, a linear relationship existed between peak blood levels and the doses injected on a body weight basis. The quotient was 1·38 ± 0·10, exceeding the value found for adult mice by 20%.

In contrast, urine levels increased much more slowly than in adults and did not form a peak. The highest values were only 15 × the blood levels (Fig. 1d), and only a fifteenth of the injected tracer was recovered in pooled urine samples collected over 3 h (Table 1).

Newborn rat. Values essentially paralleled the pattern in newborn mice (Fig. 1c and d). Blood tracer levels peaked at 1½ h, and the decline was slow. The quotient between peak levels and dose injected was 1·27. The highest urine tracer levels only exceeded blood levels 7-fold.

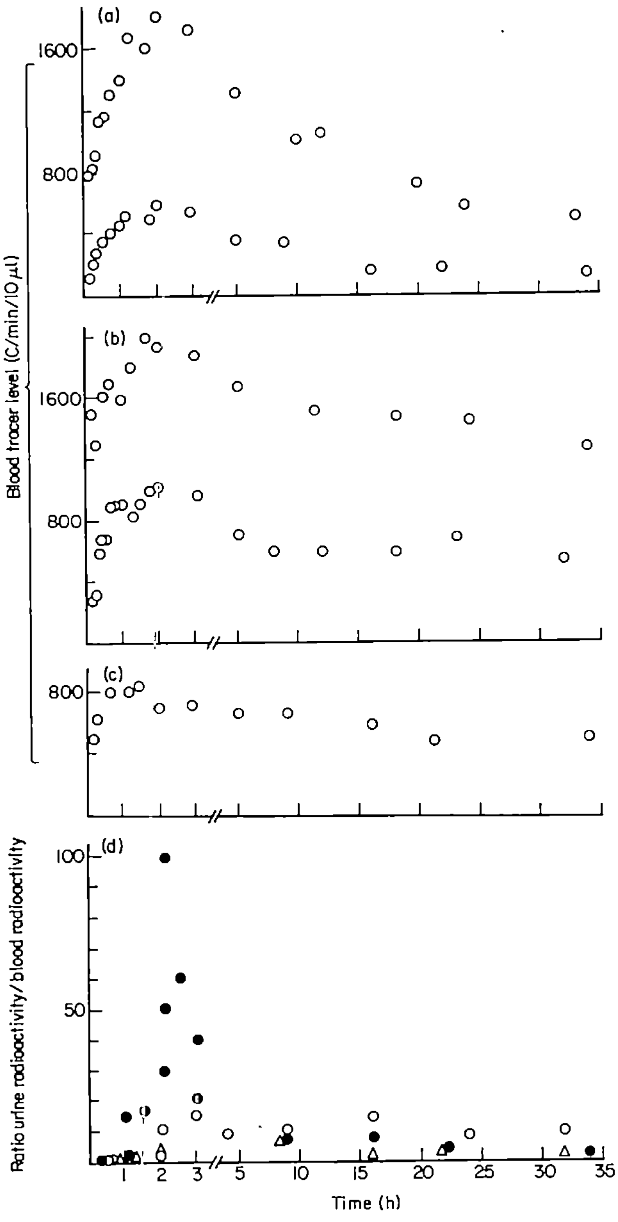


FIGURE 1. Blood tracer levels in adult and newborn mice and an adult rat after subcutaneous injection of single doses of ^{125}I-ET. Each curve represents an individual experiment. ○: single measurements.

(a) Adult mice. Doses injected: 50·000 and 150·000 c/min/g body weight.

(b) Newborn mice. Doses injected: 65·000 and 150·000 c/min/g body weight.

(c) Newborn rat. Doses injected: 65·000 c/min/g body weight.

(d) Urine tracer levels relative to blood tracer levels in adult (●) and newborn (○) mice and newborn rat (△) after subcutaneous injection of single doses of ^{125}I-ET.

Distribution of the tracer in the organism

After subcutaneous injection, I-ET is rapidly dispersed into various tissues both in adult and in newborn mice. In most organs, equilibrium between tissue and blood levels had apparently been reached by the time of the earliest measurement (30 min). The rate of increase, peak, and decline of tissue tracer was parallel to the blood tracer levels, the ratios being fairly constant throughout the observation periods.

Tissue tracer levels of both adults and newborns were generally lower than blood levels, the ratios ranging from 0·5 to 1·0 (Table 3). The kidneys, though, not only concentrated tracer relative to blood but also exhibited a significant difference of tracer accumulations between newborn and adult animals (Table 2). In adults, kidney tracer levels were highest 1 h after injection, thus preceeding the peak in the urine. In contrast, newborn kidneys accumulate only slowly.

TABLE 2. Tracer activity in kidneys

Time after injection (h)	Ratio kidney/blood tracer activity*	
	Newborn mouse	Adult mouse
0·5	0·7	Not done
1	1·0	5·1
2	1·9	3·8
4	3·7	2·1
24	5·1	1·0
48	2·1	1·0

* Single measurements.

TABLE 3. Tracer activity in various body organs

	Ratio tissue/blood tracer activity	
	Newborn mouse	Adult mouse
Liver	0·5 ± 0·16	0·5 ± 0·24
Bile	—	0·7*
Duodenum	—	0·7*
Ileum	0·5 ± 0·23	0·4 ± 0·1
Colon	—	0·4*
Spleen	0·5*	0·4 ± 0·05
Lung	0·7*	0·6*
Sternum	0·95 ± 0·07	1·4 ± 0·4
Fat	—	0·8 ± 0·3
Muscle	—	0·5*
Tongue	0·8*	0·95 ± 0·1
Skin (1 h after inj.)	0·4 ± 0·05	—
Skin (2–4 h after inj.)	1·5 ± 0·4	0·8 ± 0·2
Thyroid	1·2 ± 0·1	1·0*

These values represent the mean ratios of tissue/blood tracer activities in two to seven specimens of each organ measured.

* Only one measurement done. Specimens were taken 2–4 h after injection of I-ET, except for one measurement each of livers, skin, sternum and thyroids which were taken at 24 and 48 h.

Newborn mouse skin, in contrast to skin of adult mice, demonstrated slightly higher levels relative to blood. The thyroid glands of adults and newborns gave counts in the range of the blood tracer levels (Table 3).

Analysis of ET activity in adult mouse urine

Adult mice excrete biologically active ET, as demonstrated by the fact that newborn mouse skin is cleaved by *in vitro* incubation in the urine preparations described above to a dilution of 500 c/min/ml PBS (Table 4), as determined by (1) typical histology (Fig. 2), (2) presence of individual epidermal keratinocytes in smear preparations, and (3) peeling of the upper portions of the epidermis. This concentration corresponds to 0·5 mg crude ET/ml PBS in the crude ET-I-ET-mixture used for injection.

Control specimens incubated in the crude ET-I-ET mixture produced cleavage up to a dilution of 100 c/min/ml PBS (0·1 mg/ml).

TABLE 4. Biological ET-activity *in vitro* of dialysed adult mouse urine

Concentration of tracer (c/min/ml)	Dialysed urine	Crude ET-I-ET mixture (1000 c/min/mg crude ET)
1000	+	+
500	+	+
200	−	+
100	−	+
50	−	−

Urine was collected for 3 h after injection of crude ET-I-ET mixture (1000 c/min/mg crude ET) and processed as described in the Methods section. Newborn mouse skin was incubated 2 h at 37°C and evaluated for exfoliatin.

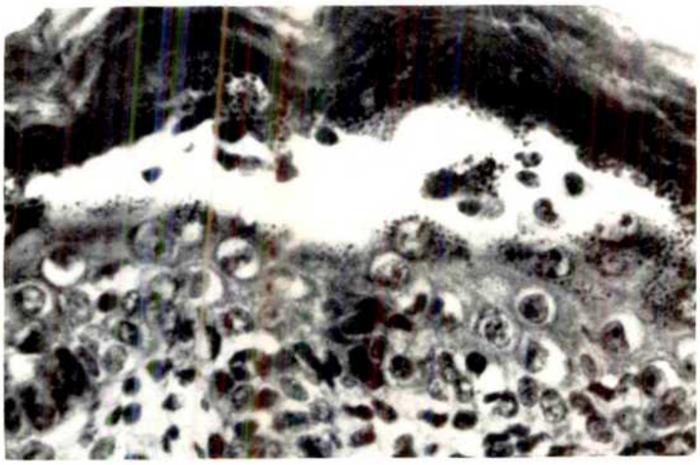


FIGURE 2. Subgranular cleavage of newborn mouse skin incubated 2 h in dialysed adult mouse urine containing 500 c/min/ml PBS tracer. Urine was collected for 3 h after injection of crude ET-I-ET mixture (1000 c/min/mg crude ET) (H & E, ×900).

Experimental generalized SSSS in adult mice

All of the four nephrectomized adult mice developed generalized SSSS 3–4 h after intramuscular injection of ET as documented by a positive Nikolski sign (Fig. 3), histology and smear preparations. Sham-operated animals injected with ET, as well as nephrectomized animals not injected with ET, did not develop SSSS.

The three subtotally hepatectomized adult mice injected with comparable doses of ET did not develop generalized SSSS.

Two of the four carbon tetrachloride-treated animals developed severe generalized SSSS 4 h after intraperitoneal injection of ET. These two animals had appeared much sicker prior to injection of ET than the others and showed much more extensive liver and kidney damage histologically (see below). Carbon tetrachloride-poisoned animals not injected with ET as well as animals given mineral oil without carbon tetrachloride, followed by ET-injection, did not develop generalized SSSS.

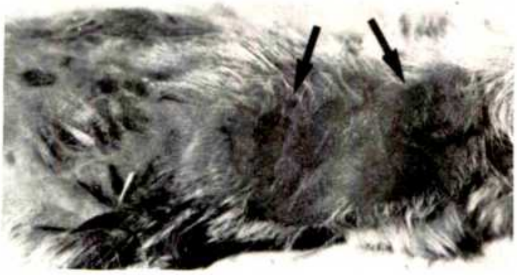


FIGURE 2. Generalized SSSS in nephrectomized adult mouse 4 h after administration of 20 mg crude ET intramuscularly into thigh. Note positive Nikolski phenomenon in interscapular area (arrow).

Histologically, the livers of adult mice treated with carbon tetrachloride 20 h previously showed marked centrolobular eosinophilic necrosis as well as peripheral fatty degeneration at 20 h after administration. At 72 h, a sharp demarcation of the central necrotic areas from surrounding more normal regions was found. The kidneys showed extensive degeneration and necrosis of proximal and distal renal tubules as well as of renal papillae. Carbon tetrachloride-treated animals exhibiting generalized SSSS after ET-injection showed identical changes in their livers and kidneys. Finally, animals given comparable doses of ET without prior poisoning, demonstrated no hepatic or renal abnormalities.

DISCUSSION

Generalized SSSS in relation to renal function

The data presented reveal a significant difference in the excretion patterns of ET between newborn and adult mice. In adult mice renal excretion of a single dose is rapid, the bulk of the material being eliminated within a few hours. In contrast, newborns excrete ET slowly. The blood levels, therefore, rise somewhat higher than in adults, and the decline of the curve is much slower. Thus it can be expected that, with continuous administration, ET will be rapidly accumulated in the organisms of newborn mice whereas adults are equipped to deal with much higher amounts of ET.

Since ET is water soluble with an approximate molecular weight of 25,000 daltons, passage through the renal glomeruli is likely to be unimpeded. The excretion rate of ET, therefore, would be proportional to the glomerular filtration rate. It is well documented that the kidneys of a variety of mammals, including humans, are morphologically and functionally immature at birth with regard to such parameters as glomerular filtration rate, tubular transport activity, water excretion and renal concentration capacities. Depending on the species, several weeks and periods up to 2 years (as is the case in man) are needed to achieve renal function equal to that of the adult organism (McCroy, 1972). Although, to our knowledge, no data are available on kidney function of newborn mice, it can be assumed that they follow the same pattern and that delayed excretion of ET by newborns is a reflection of immature renal function. Quite obviously, the differences in excretion rates of ET between newborns and adults follows a more general rule pertaining to most or all mammals and most or all substances administered of comparable size and structure. It is, therefore, not surprising that blood levels and excretion patterns after injection of ^{125}I-ribonuclease into newborn and adult mice very closely resembled those observed with I-ET (unpublished observation). Furthermore, newborn rats, although belonging to a species non-susceptible to ET (Elias, Fritsch & Mittermayer, 1976), showed an almost identical distribution and excretion pattern of I-ET to the susceptible newborn mice. The maturity of kidney function, therefore, seems to be an important factor for determining whether threshold levels for exfoliatin are reached in the epidermis thereby conveying the resistance of adults to generalized SSSS. On the other hand, renal function does not explain species resistance to ET which is likely to be a genetically determined characteristic of the epidermis itself (Elias *et al.*, 1976).

A possible exception to the rule of resistance of adults to generalized SSSS has been reported in hairless mice by Arbuthnott *et al.* (1973). Since no distinction was made in this study between localized and generalized SSSS, an interpretation of these findings is difficult at the present time.

Possible extrarenal metabolism of ET

Several observations, however, suggest that the mechanisms involved may be more complex. Although a portion of ET excreted into the urine by adult mice retains biological activity, total potency of the urine is decreased to a fifth. This difference probably cannot be fully explained by loss of activity in the moderately acid urine (pH 6·0–6·5) or by inherent inaccuracies of the assay. Furthermore, a 10–20-fold dose of ET on body weight basis should compensate for more rapid renal excretion in adult mice and lead to generalized SSSS. Since this is not the case, catabolism of ET in the adult organism is likely. This view is corroborated by preliminary results of isoelectric focusing studies which indicate that I-ET is split into four radioactive bands in the urine of the adult mouse, only one of which is identical with the band of the purified native ET (Fritsch, Elias & Varga, 1976).

Presumably, transformation and partial inactivation may occur in the liver although some participation of the renal tubules cannot be excluded.

Our experiments do not allow a clear conclusion as to the role of the liver in the fate of ET. The low tracer levels in bile and duodenum prove that ET is not preferentially excreted via the biliary system, but the absence of ET accumulations in the liver does not necessarily exclude some important metabolic activities. However, subtotally hepatectomized mice did not undergo generalized SSSS when injected with ET.

Carbon tetrachloride poisoning was performed in lieu of total removal of the liver since the animals did not survive long enough to be evaluated for SSSS. Again, a clear-cut answer was not found since carbon tetrachloride inflicted both hepatic and renal damage; animals with partial liver necrosis did not respond to ET and those exhibiting generalized SSSS suffered from severe both kidney and hepatic necrosis.

The tracer was not accumulated in any of the body organs measured either in newborn or adult mice. This was particularly interesting with regard to the thyroid glands indicating that no significant amounts of free ^{125}I were formed in the course of possible degradation of I-ET. Also, whole skin did not accumulate I-ET to any great extent although the values for newborn skin were among the highest found. Skin tracer activity included the dermal tracer activity which probably made up for the major part because of the higher relative mass of the dermis and its blood content. Binding of I-ET to the epidermis therefore may still have occurred.

More detailed studies are currently under way to determine the role of the liver in the metabolism of ET. The importance of the kidneys in the development of generalized SSSS, however, is obvious. Whether metabolically compromised adults will prove to be at increased risk for development of SSSS is still not known. However, at least two of the adult cases reported to date did have renal insufficieny (Levine & Norden, 1972; Reid *et al.*, 1974). Our studies thus provide a rationale for the age distribution of SSSS in humans as well as in mice and may explain why neonates are more likely to develop SSSS than adults.

ACKNOWLEDGMENTS

This study was supported by a Fellowship Grant of the Max Kade Foundation to Dr P.Fritsch and by USPHS grant CA-15557 and NSF grant GM-12607 to Dr J.Varga.

REFERENCES

Arbuthnott, J.P., Kent, K., Lyell, A. & Gemmel, C.G. (1972) Studies on staphylococcal toxins in relation to toxic epidermal necrolysis (the scalded skin syndrome). *British Journal of Dermatology*, **86**, supplement 8, 35.

Arbuthnott, J.P., Kent, J. & Noble, W.C. (1973) The response of hairless mice to staphylococcal epidermolytic toxin. *British Journal of Dermatology*, **88**, 481.

Awdeh, Z.L., Williamson, A.R. & Askonas, B.A. (1968) Isoelectric focusing in polyacrylamide gel and its application to immunoglobulins. *Nature*, **219**, 66.

Elias, P.M., Mittermayer, H., Tappeiner, G., Fritsch, P. & Wolff, K. (1974) Staphylococcal toxic epidermal necrolysis (TEN): the expanded mouse model. *Journal of Investigative Dermatology*, **63**, 467.

Elias, P.M., Fritsch, P., Dahl, M.V. & Wolff, K. (1976) Staphylococcal toxic epidermal necrolysis: pathogenesis and subcellular site of action. *Journal of Investigative Dermatology*, **65**, 501.

Elias, P.M., Fritsch, P. & Epstein, E. (1976) Staphylococcal scalded skin syndrome: a review. *Archives of Dermatology*, in press.

Elias, P.M., Fritsch, P. & Mittermayer, H. (1976) Staphylococcal toxic epidermal necrolysis: species and tissue susceptibility and resistance. *Journal of Investigative Dermatology*, **66**, 80.

Epstein, E.H. Jr., Flynn, P. & Davis, R.S. (1974) Adult toxic epidermal necrolysis with fatal staphylococcal septicemia. *Journal of the American Medical Association*, **229**, 425.

Fritsch, P. (1975) Staphylogene toxische epidermale Nekrolyse. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **50**, 5.

Fritsch, P., Elias, P.M. & Varga, J. (1976) In preparation.

Greenwood, F.C., Hunter, W.M. & Glover, J.S. (1963) The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochemical Journal*, **89**, 114.

Hawley, H.B. & Aronson, M.D. (1973) Letter to the Editor. *New England Journal of Medicine*, **228**, 1130.

Kapral, F.A. & Miller, M.M. (1972) Skin lesions produced by *staphylococcus aureus* exfoliatin in hairless mice. *Infection and Immunity*, **6**, 877.

Levine, G. & Norden, C.W. (1972) Staphylococcal scalded skin syndrome in an adult. *New England Journal of Medicine*, **287**, 1339.

McCroy, W.W. (1972) *Developmental nephrology*. Harvard University Press.

Melish, M.E. & Glasgow, L.A. (1970) The staphylococcal scalded skin syndrome: development of an experimental model. *New England Journal of Medicine*, **282**, 114.

Melish, M.E. & Glasgow, L.A. (1972) The staphylococcal scalded skin syndrome. Isolation and partial characterization of the exfoliative toxin. *Journal of Infectious Diseases*, **125**, 129.

Reid, L.H., Weston, W.L. & Humbert, J.R. (1974) Staphylococcal scalded skin syndrome. *Archives of Dermatology*, **109**, 239.

Ritter von Rittershain, G. (1975) Die exfoliative Dermatitis jüngerer Säuglinge. *Centralzeitung für Kinderheilkunde*, **2**, 3.

Rothenberg, R., Renna, F.S., Drew, T.M. & Feingold, D.S. (1973) Staphylococcal scalded skin syndrome in an adult. *Archives of Dermatology*, **108**, 408.

Tachau, P. (1934) Pemphigoid der Neugeborenen und Kinder. In: *Handbuch der Haut- und Geschlechtskrankheiten* (Ed. by J.Jadassohn) IX/2, pp. 159–234. Springer, Berlin.

Varga, J.M., Dipasquale, A., Pawelek, J., McGuire, J.S. & Lerner, A.B. (1974) Regulation of melanocyte stimulating hormone action at the receptor level: discontinuous binding of hormone to synchronized mouse melanoma cells during the cell cycle. *Proceedings of the National Academy of Science*, **71**, 1590.

British Journal of Dermatology (1976) **95**, 285.

Hypersensitivity to bacteria in eczema

II. TITRE AND IMMUNOGLOBULIN CLASS OF ANTIBODIES TO STAPHYLOCOCCI AND MICROCOCCI

W.E.PARISH,* ESTHER WELBOURN AND R.H.CHAMPION

The Lister Institute of Preventive Medicine, Elstree, Herts.,* and
Department of Dermatology, Addenbrooke's Hospital, Cambridge

Accepted for publication 16 December 1975

SUMMARY

The amounts of agglutinating antibody to staphylococcal and micrococcal phenol-extracted (probably teichoic acids) and protein antigens, prepared from Baird Parker types SI, SIV and MI, were no greater in eczematous than in control persons. Most antibody to phenol-extracted antigens was IgM which frequently activated complement to lyse red cells which had adsorbed the bacterial antigen. IgG antibody against phenol-extracted staphylococcal antigen (*Staph. aureus*, Baird Parker type SI) was shown to be specific antibody combining by the $F(ab)_2$ portion of the molecule. The phenol-extracts did not contain staphylococcal Protein A that binds to the Fc portion of IgG. Furthermore, the presence of Protein A in the protein or total antigen extracts did not appear to modify the results of the tanned cell agglutination test.

The occurrence of immediate or of 4 h, Arthus-like skin test responses to staphylococcal or micrococcal antigens was unrelated to the agglutinin or complement-lysis titre of the relevant antibody.

In a study of the cytotoxic effect of antibacterial antibody reacting with bacterial antigen acquired by epidermal cells, we reported the results of bacterial culture, of skin tests, and of immunofluorescence tests for immunoglobulins, complement and bacterial antigens in normal and diseased skin of patients with disseminated eczema (Welbourn, Champion & Parish, 1976a).

We now report the results of tests to detect antibacterial antibodies in 87 of the 122 patients, in 17 controls to the patients (wife or husband), and in a further 118 normal healthy persons. The immunoglobulin class of the antibodies was determined and their ability to activate complement.

MATERIALS AND METHODS

The patients and the preparation of the bacterial antigens have already been described (Welbourn

---

* Present address: Unilever Research, Colworth House, Sharnbrook, Bedford.

et al., 1976a). The phenol extract contained 7·2% phosphate and precipitated with 1% DEAE-dextran in agarose gels (Dudman, 1972).

The *Staphylococcus aureus* was a typical coagulase positive Baird Parker type SI. The organism described as the micrococcus FI in these reports was a Baird Parker type SVI, and that described as MI was a Baird Parker type MI. All were obtained from eczematous patients.

The culture of *Staph. aureus* formed little Protein A, on the evidence that it was difficult to obtain a gel plate precipitin line against human sera, compared to the ease with which such lines were obtained by extracts of other cultures against the same sera.

Further control sera were obtained from 118 normal persons, including healthy persons before the injection of bacterial antigens, blood donors and persons whose sera were being assayed for concentrations of immunoglobulins. Their ages were from 15 to 82 years.

Tests for antibodies to bacterial antigens

Antibodies to phenol-extracted antigens were detected by sheep cells treated with the antigenic solution.

Sheep red cells were incubated for 3 h in 0·1% antigen solution, were washed thrice and made up as a 1% suspension in phosphate buffered saline. The test sera were heated at 56°C for 30 min, adsorbed with normal sheep red cells, and were titrated by doubling dilutions in 0·1 ml volumes in saline: 0·1 ml of the antigen-treated sheep cells was added to each tube. A control positive, and negative serum was included in each test. Complement-lysis tests were made by adding 0·1 ml of $3CH_{50}$ baby guinea-pig serum, previously absorbed at 2°C with antigen treated sheep cells, to each tube after an agglutination test, or occasionally in repeat tests, adding the complement with the antigen-treated red cells. The tubes were kept overnight at 4°C, and incubated for 30 min at 37°C before examining for lysis.

Antiglobulin tests were always made on repeat agglutination tests using 0·2 ml of serum and of antigen-coated red cells. (The heavier cell deposit results in lower direct agglutination titres). After the cells had agglutinated or settled, the serum was removed from each tube and the cells dispersed in and washed thrice in 0·5 ml saline, finally being resuspended as 0·2 ml. The washed cells were then dispensed by drops into rows of monospecific anti-IgG, -IgA, -IgM and of normal (no antiglobulin) serum control, all suitably diluted according to their optimal antiglobulin titre (Parish, 1969). An antiglobulin titre was recorded when the titre for that antiglobulin serum (e.g. anti-IgG) was at least one tube greater than that occurring in the normal serum control, or when in doubt above that of the direct agglutination titre. That is, the antiglobulin serum had to agglutinate the antigen-treated red cells, which had the patient's antibody (e.g. IgG) fixed to them, to a higher titre than occurred by direct agglutination.

Antibodies to the protein antigens were detected by the tanned red cell agglutination test of Boyden (1951) slightly modified (Gunther *et al.*, 1960). The concentration of protein used to treat the tanned red cells was 0·5% of the purified extract, or that contained in the total extract.

Preparation of IgG Fc and pFc fragments

In order to ascertain whether or not the agglutination occurring in tests with *Staph. aureus* antigen was mediated by specific antibody, or by staphylococcal Protein A that combines with IgG Fc, IgG was separated from sera of two eczematous and three normal persons. It was treated with pepsin as described by Stanworth & Turner (1973) and the pFc and $F(ab)_2$ portions separated on Sephadex G150. IgG from another two sera from eczematous patients and two normal sera were treated with papain by the method of Sanderson & Lanning (1970) to obtain the Fc and Fab portions. Fractions containing the fragments were concentrated to the amounts which would have been present in the original whole IgG assuming that none had been lost.

RESULTS

The sera of eighty-seven of the 122 eczematous patients, some from each G, N, O group, were examined for anti-bacterial agglutinins. Agglutinin titres to phenol-extract antigens of *Staph. aureus* and micrococcal cultures were mainly between 2 and 16, but many of these antibodies failed to lyse the agglutinated antigen-adsorbed red cells on subsequent incubation with complement (Table 1).

TABLE 1. Anti-bacterial antibodies in sera of eighty-seven eczematous patients

	No. of persons with titres of antibodies mediating						
	Staph. aureus			*Micrococcus Ml*		*Micrococcus F*	
Titre	Aggl.	C-lysis	TCT	Aggl.	C-lysis	Aggl.	C-lysis
0	23	63	37	23	75	15	77
2	11	5	8	8	4	8	5
4	12	6	5	20	2	19	1
8	14	4	6	14	3	13	2
16	13		8	10	1	19	1
32	4	2	2	4		8	
64	1	4	2	6	2	3	
128	2	1	10	1		1	1
256	3	1	4	1		1	
512	2	1	1				
1000	2		4				

Aggl = agglutination of phenol-extracted antigen adsorbed onto sheep red cells.

C-lysis = lysis on adding complement to the antigen-coated red cells agglutinated by the patient's serum.

TCT = tanned cell agglutination. Tannic acid-treated sheep red cells adsorbing whole, protein-containing, extract of *Staph. aureus*.

Only fourteen of the eighty-seven persons had agglutinin titres between 32 and 1000; these titres were not associated with current infection. Titres above 32 were found in twenty-three of the eighty-seven patients when sera were tested against total extract of the staphylococcal organisms by the tanned red cell agglutination test. The total extract contained proteins and some nucleic acids absent from the phenol extracts. More persons with a history of recent staphylococcal infection, e.g. a boil, had agglutinin titres greater than 32 against total extract than against phenol-extracted antigens.

The titres of antibodies to *Staph. aureus* in the sera of the eighty-seven eczematous patients were not significantly greater than in the seventeen control persons of this study, and in 118 normal persons, when the percentages of sera having one of three ranges of titre were compared (Table 2). The agglutinin titres of the husband or wife controls were usually as great or even greater than those of the eczematous married partners sharing the same environment.

The mean agglutinin titres to micrococcal culture Ml and to culture F, in eczematous, control and normal persons were very similar. These results, however, do not indicate close antigenic similarity

TABLE 2. Percentages* of eczematous patients, control, and normal persons in each of three ranges of titres of antibacterial agglutinins and complement-lysing antibodies

Sera (no. & origin)	Titre	*Staph. aureus*			*Micrococcus Ml*		*Micrococcus F*	
		Aggl.	C-lysis	TCT	Aggl.	C-lysis	Aggl.	C-lysis
87 eczematous patients	0–4	53	85	58	59	93	48	95
	8–64	37	12	21	40	7	50	4
	128–1000	10	3	22	2	0	2	1
17 controls patients	0–4	73	93	60	60	100	60	100
	8–64	20	7	20	40	0	40	0
	128–1000	7	0	20	0	0	0	0
118 normal persons	0–4	77	80	53	55	96	51	99
	8–64	18	17	25	44	4	48	0
	128–1000	5	3	22	1	0	1	1

* Percentages to nearest whole number.

in the two micrococcal cultures, because some sera had antibody to one culture only, and in many other sera the antibody titre to Ml differed from that of F.

Antiglobulin agglutination tests

The sera of another twenty-seven eczematous patients were examined for the classes of immuno-

TABLE 3. Anti-bacterial antibodies in sera of eczematous patients and normal persons detected by antiglobulin agglutination and complement lysis. Number of persons with titres of agglutinating or complement-lysing antibodies to phenol-extracted antigens adsorbed onto sheep erythrocytes

Bacterial antigen	Titres	Eczematous patients (27 sera)				Normal persons (92 sera)		
		IgG	IgA	IgM	C-lysis	IgG	IgA	IgM
Staph. aureus	0	13	20	6	14	41	62	7
	2–8	2	1	8	5	36	19	44
	16–64	8	6	12	8	11	8	41
	128–2000	4	0	1	0	4	3	0
Microc. Ml	0	17	27	11	17	53	64	38
	2–8	4		9	6	31	28	25
	16–64	4		7	3	8	0	29
	128–2000	2				0	0	0
		(12 sera)						
Strep. viridans	0	4	11	3	6	21	44	31
	2–8	1	1	8	4	30	42	43
	16–64	4		1	2	35	6	18
	128–2000	3				6	0	0

Titres of antiglobulin tests are those greater than the titre of agglutination in the normal serum control. Antiglobulin titres equal to that of the control serum are reported as 0 (see text).

globulin agglutinins against phenol-extracted *Staph. aureus* and micrococcus Ml antigens, and twelve of the twenty-seven sera were similarly examined for antibodies to *Streptococcus viridans*. The titres of the antiglobulin tests (Table 3) are those in which the monospecific anti-IgG, -IgA or -IgM agglutinated the antigen-treated cells fixing patient's antibody in at least one dilution greater than that occurring in the normal serum (no antiglobulin) control, as described in Methods. Thus, amounts of specific antibody in the patient's serum, too small to combine with the bacterial antigen to bring about agglutination when subsequently treated with the particular antiglobulin serum, are not detectable by this method and are recorded as zero (Table 3). In several sera the same titre was observed in tests with each of the three antiglobulins and the normal serum control, e.g. all four titres were 1/8. Such a result is recorded as zero in Table 3. It appears to reflect the presence of a low titre of very efficient IgM antibody that agglutinates the antigen coated cells, without enough molecules binding to the cell for the agglutination to be augmented by anti-IgM sera. IgM rarely occurs in an 'incomplete' non-agglutinating form as does IgG.

It is evident that most of the antibody was IgM, though some sera contained both IgG and IgM antibodies. IgM antibodies mediated most of the complement lysis in the ranges of titres 2–8 and 16–64, though there were several sera with IgM antibodies that did not activate complement. IgA antibody in titres of 16–64 was found in six sera with antibody to *Staph. aureus*. No IgA antibody to Micrococcus Ml antigen was found and only one person had IgA anti-*Strep. viridans*, with a low titre.

Similar results were obtained in antiglobulin agglutination tests on sera of ninety-two normal persons (Table 3). Most of the antibody to the staphylococcal and micrococcal antigens was IgM, but more sera had IgG than IgM antibodies to the streptococcal antigen. A greater proportion of sera from the twenty-seven eczematous patients had titres of 128 or more, than did sera from the normal persons.

The results of tests with antigens of micrococcus F culture and of *Esch. coli* somatic groups 111 and 124 do not contribute more to the above findings.

Results of pepsin and papain hydrolysis of IgG anti-staphylococcal antibodies

In order to confirm that the antiglobulin tests on patient's sera for IgG antibody detected specific antibody combining with the antigen by $F(ab)_2$, and not non-specific adherence of Fc, IgG fractions of two eczematous, and three normal persons were hydrolysed by pepsin, and the digest separated on Sephadex G 150. Each IgG fragment was tested on staphylococcal antigen-treated red cells (as for the agglutination tests). The cells were then washed, and tested with anti-IgG Fc and anti-Fab to determine which fractions bound in sufficient amount to the red cells to be agglutinated by the appropriate antiglobulin fragment antisera. Agglutination of the cells by anti-Fab antibody indicates binding by specific anti-staphylococcal antibody; agglutination by anti-Fc would indicate antigen non-specific binding by the patient's IgG.

Similar tests were made on IgG, from two eczematous and two normal persons, hydrolysed by papain to obtain the Fc and Fab portions.

Antiglobulin agglutination occurred only when the antigen-treated red cells were tested with the $F(ab)_2$ and Fab fragments and subsequently with anti-Fab antiserum. The antigen-treated red cells either did not adsorb, or adsorbed too little of the Fc to be agglutinated by the anti-Fc antiserum (Table 4).

The results of the antiglobulin agglutination tests for the antibacterial antibodies in the sera of the patients therefore reflect the activity of specific antibody, and not of non-specific adherence of IgG.

During these tests on the fragments of IgG it became evident that the procedures of preparation could modify the results, mainly depending upon the duration of enzyme hydrolysis. With longer

TABLE 4. Titres of antiglobulin tests with anti-IgG Fc and anti-Fab on pepsin and papain-cleaved fragments of IgG antibody to phenol-extracted staphylococcal antigen adsorbed to sheep red cells

Hydrolysing enzyme	Donor of serum	Whole* IgG anti-Fc	pFc or Fc† fragments		F(ab)$_2$ or Fab† fragments	
			Anti-Fc	Anti-Fab	Anti-Fc	Anti-Fab
Pepsin	Eczema	8	0	0	0	4
	Eczema	16	0	0	0	4
	Eczema	64	0	0	0	32
	Normal	4	0	0	0	2
	Normal	32	0	0	0	8
Papain	Eczema	32	0	0	0	8
	Eczema	128	0	0	0	32
	Normal	16	0	0	0	16
	Normal	16	0	0	0	8

* Antibody titres in fraction of whole IgG separated from serum by DEAE cellulose.

† Antibody titres in pFc and F(ab)$_2$ fragments (pepsin hydrolysis) and Fc and Fab fragments (papain hydrolysis).

times of hydrolysis, the specific activity of the F(ab)$_2$ or Fab was reduced, and some binding by Fc (papain) could occur. Furthermore, in preliminary tests in which Fc and pFc were labelled with radioactive iodine, some binding to the antigen-treated red cells was seen, even though the anti-Fc antiserum did not agglutinate the cells. Details of these findings will be reported separately.

Validity of using one antigen preparation of Staph. aureus

It is possible that antibody titres to the antigens of the one culture of *Staph. aureus* used in this study do not truly represent the titre of antibody formed by the patient against the staphylococci of his own skin. This possibility was examined by extracting antigens by phenol or by a freeze-press from staphylococci obtained from the skin of twelve patients, and testing the antigens of each culture against all twelve sera. The phenol-extracted antigens of each culture gave consistent results with all the sera, the variation not being more than one antibody dilution above or below that of the serum of the donor of the culture. The freeze-press total extracts in the tanned red cell agglutination test were more variable. One culture in particular tended to give higher titres than the others. The antigens of the one culture of *Staph. aureus* are therefore considered to give a good indication of the antibody titres to phenol-extracted antigen, and a reasonable indication of the antibody titres to the total extracts.

Relation between titre of anti-bacterial antibody and skin test responses to the bacterial antigens

There was no relation between the immediate and Arthus or other 4-h skin-test responses to the particulate or soluble extracted antigens, and the titre of agglutination, the complement-lysis tests or the class of immunoglobulin agglutinins. The phenol-extracted antigens and the total, protein-containing, extracts used in the skin tests were the same as those used in the serological tests.

No *in vitro* tests were made to detect IgE antibodies to account for the immediate reactions, but no IgE anti-staphylococcal or micrococcal antibodies were found by monkey passive cutaneous anaphylaxis on fifty-two samples.

Arthus-type or 4-h skin-test responses occurred as frequently in persons whose sera had low titres of agglutinins, and no complement activation, as in those with higher titres of antibodies which activate complement. Moreover, some persons with agglutinin titres above 128 to staphylococcal phenol-extracted antigen or to protein, gave no skin test response to these antigens.

The titre of agglutination considered sufficient to mediate an Arthus response was 16, and of complement lysis, 4, based on previous experience of intracutaneous skin test reactions with a range of dilutions of egg albumen, and pollen antigen in persons sensitized to these antigens. The occurrence of 4-h, Arthus-like responses related to antibody titre to the eliciting antigen, i.e. phenol extract or protein (total) extract of *Staph. aureus*, or micrococcus Ml, was:

4-h response. Agglutinin titre over 16; complement-lysis 4 or over, in twenty-six persons.

4-h response. Agglutinin titre over 16; complement-lysis absent or less than 4, in three persons.

No 4-h response. Agglutinins absent or less than 16; complement-lysis absent or less than 4, in twenty-seven persons.

No 4-h response. Agglutinin titre over 16; complement-lysis 4 or over, in six persons.

DISCUSSION

It is evident from these findings that the amount of antibody to staphylococcal and micrococcal antigens is no greater in eczematous persons who have increased numbers of staphylococci and micrococci on their skin, than in normal persons, including the normal husband or wife living in the same environment as the eczematous patient.

Antibodies of immunoglobulin classes G, M and A were detected against phenol-extracted antigens of *Staph. aureus* (Baird Parker type Sl), and micrococci (Baird Parker types S IV and Ml).

Much of the phenol-extracted antigen was probably teichoic acid, with a small proportion of nucleic acids. The identity of the antigens in the extracts still needs to be determined, but the method of preparation with hot phenol and precipitation with 70% alcohol, the high phosphate content of the extracts, 7·2%, the precipitation with 1% DEAE-dextran, and the biological activity, e.g. spontaneous absorption to red cells, are strong presumptive evidence. Teichoic acids are a major constituent of the cell walls of *Staph. aureus* and several other Gram positive bacteria (Armstrong *et al.*, 1958). The firm spontaneous adsorption to red cells enables the cells to be used in haemagglutination tests to detect anti-teichoic acid antibodies (Brock & Reiter, 1971) and as used in our investigation.

The antibodies to the phenol-extracted antigens were mainly IgM, which mediated most of the complement-lysis of the antigen-treated red cells. It was difficult to identify the IgM antibodies by the antiglobulin agglutination tests, because IgM is an extremely potent agglutinin, and seldom occurs in an incomplete form. Thus in an anti-globulin agglutination test, the IgM antibodies remain firmly attached to the antigen during washing, and agglutinate the cells again when samples are placed in the various antiglobulin sera. The same titre is therefore seen in the control tubes containing normal serum as in the other tubes containing the monospecific antiglobulin sera to IgG, IgA and IgM. The anti-IgM does not enhance agglutination in this system as does anti-IgG, or anti-IgA in similar tests with protein antigens, when the antiglobulin titres are considerably greater than that of the control, non-antiglobulin serum (Parish, 1969).

Some sera had IgG antibodies to the phenol-extracted antigens, which did not activate complement to lyse antigen-treated red cells. In later tests, four such samples were shown to have IgG_2 subclass antibodies, and in two sera with higher titres of IgG antibody which activated complement, there were also IgG_1 subclass antibodies. These results, which will be reported separately, are consistent with the findings in persons forming antibodies to carbohydrate-associated antigens in bacterial vaccines, e.g. typhoid and cholera vaccines (Parish, 1975).

The complete lack of correlation between the skin test responses and occurrence of complement-activating antibodies to the test antigens is of particular interest. The occurrence of immediate reactions of patients to the bacterial antigens in 21·8–75% of the three groups (Welbourn *et al.*, 1976a) was unrelated to the presence or titre of antibody.

A classical immediate weal and flare response is mediated by IgE antibody, but no such antibacterial antibody was found in fifty-two samples by monkey passive cutaneous anaphylaxis. This, however, is not a very discriminating technique for IgE.

Four-hour Arthus-like reactions occurred almost equally in persons with or without complement-activating antibody. The failure to elicit Arthus-like responses in six persons considered to have sufficient antibody, could be due to an inappropriate amount of antigen in the skin test. Arthus reactions occur with antigen in slight to moderate excess of equivalence of antibody.

In our study of cytotoxicity, it was not essential to use pure preparations of antigen to examine the allergic state of the patients. However, in a critical study of the relation of antibody to the Arthus-type response, it would be essential to use pure preparations of single bacterial antigens for the skin and *in vitro* tests.

Another difficulty in the interpretation of skin test responses to bacteria results from the formation by *Staph. aureus*, and some other bacteria, of a substance designated Protein A. This protein combines without antigen specificity with the Fc portion of human IgG to form a complex that has several properties of specific antigen-antibody complexes. The complex of Protein A and Fc of IgG forms precipitates, agglutinates Protein A containing particles, and induces immediate, anaphylaxis-like, and 4-h, Arthus-like skin responses (Forsgren & Sjöquist, 1966; Sjöquist & Stalenheim, 1969; Gustafson *et al.*, 1967, 1968).

The results of the skin tests and agglutination tests in our study are not complicated by the activity of Protein A. The *Staph. aureus* culture used to prepare the antigens formed Protein A, though not in large amounts. Protein A was absent from the phenol-extracted antigen (teichoic acids) used for both skin and agglutination tests. Though Protein A was present in the total (freeze-press) extracts containing protein, it did not appear to modify the tanned cell agglutination test results, because less than 50% of the sera agglutinated the red cells; if the agglutination was modified by Protein A, nearly 100% of the sera would have agglutinated the cells.

The same lack of relation between the results of the skin tests and *in vitro* antibody tests occurred with the micrococcal antigens, but the micrococci do not synthesize Protein A. These results are discussed in more detail in the succeeding report (Welbourn, Champion & Parish, 1976b).

Furthermore, we showed that the specific antigen-binding portions of the IgG molecule, the $F(ab)_2$ or Fab, and not the Fc portion, combined with the teichoic acid-treated red cells. This finding was consistent in the sera from four eczematous and five normal persons.

The finding of IgM complement-activating antibodies, and in a few instances IgG antibodies, to phenol-extracted antigens, probably teichoic acids, of staphylococci and micrococci, is further evidence that eczematous cytotoxic changes may be induced by these antibodies reacting with epidermal cells acquiring the bacterial antigens. This activity of the antibodies *in vitro* is shown in a subsequent paper (Parish, Welbourn & Champion, 1976).

ACKNOWLEDGMENTS

We are grateful to the Smith Kline & French Foundation for research grants to E.W. and W.E.P., and also to the Addenbrooke's Hospital Research Fund for a further grant to E.W., without whose help this and related long-term studies could not have been completed.

We are grateful for advice and support from Drs Arthur Rook and Granville R.E.Naylor, and also to Drs Arthur Rook and Stephen Roberts for referring patients for the investigation. We thank Dr

M.H.Gleeson-White, Mr S.T.Haslam and the Bacteriological Laboratory for the identification of the bacteriological cultures, and the following staff of the Lister Institute for much careful technical assistance—Miss Marisa Walisiewick, Mrs Gillian Wardle, Mrs Geraldine Yates, Mr Kenneth Veal, Mr Michael Scott and Miss Susan Hedges. We thank the Junior Staff of the Dermatology Department, Addenbrooke's Hospital for their help, and we especially thank the patients and the controls for their co-operation for so many years.

REFERENCES

ARMSTRONG, J.J., BADDILEY, J., BUCHANAN, J.G., CARSS, B. & GREENBERG, G.R. (1958) Isolation and structure of ribitol phosphate derivatives (teichoic acids) from bacterial cell walls. *Journal of Chemical Society*, p. 4344.

BOYDEN, S.V. (1951) The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by anti-protein sera. *Journal of Experimental Medicine*, **93**, 107.

BROCK, J.H. & REITER, B. (1971) Sensitisation of sheep erythrocytes by cell wall teichoic acid of *Staphylococcus* aureus. *Immunochemistry*, **8**, 933.

DUDMAN, W.F. (1972) Detection of acidic polysaccharides in gels by DEAE-dextran. *Analytical Biochemistry*, **46**, 668.

FORSGREN, A. & SJÖQUIST, J. (1966) Protein A from S. aureus. I. Pseudo-immune reaction with human gamma-globulin. *Journal of Immunology*, **97**, 822.

GUNTHER, M., ASCHAFFENBURG, R., MATHEWS, R.N., PARISH, W.E. & COOMBS, R.R.A. (1960) The level of antibodies to the proteins of cow's milk in the serum of normal infants. *Immunology*, **3**, 296.

GUSTAFSON, G.T., SJÖQUIST, J. & STALENHEIM, G. (1967) 'Protein A' from *Staphylococcus aureus*. II. Arthus-like reaction produced in rabbits by interaction of Protein A and human γ globulin. *Journal of Immunology*, **98**, 1178.

GUSTAFSON, G.T., STALENHEIM, G., FORSGREN, A. & SJÖQUIST, J. (1968) 'Protein A' from *Staphylococcus aureus*. IV. Production of anaphylaxis-like cutaneous and systemic reactions in non-immunized guinea-pigs. *Journal of Immunology*, **100**, 530.

PARISH, W.E. (1969) Detection of reagins, IgG, IgA and IgM antibodies in human sera. *International Archives of Allergy and Applied Immunology*, **36**, 245.

PARISH, W.E. (1975) Some biological activities of IgG, subclass antibodies after inoculation and in disease. In: *Allergy '74. The proceedings of the 9th European Academy of Allergology and Clinical Immunology* (Ed. by M.A.Ganderton and A.W.Frankland), p. 153. Pitman Medical Press, London.

PARISH, W.E., WELBOURN, E. & CHAMPION, R.H. (1976) Hypersensitivity to bacteria mediating cytotoxic changes in generalised eczema. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens. *British Journal of Dermatology*, **95**, in press.

SANDERSON, A.R. & LANNING, M.G. (1970). Crystalline Fc prepared in high yield from normal human IgG. *Nature (London)*, **226**, 356.

SJÖQUIST, J. & STALENHEIM, G. (1969) Protein A from *Staphylococcus aureus*. IV. Complement-fixing activity of protein A-IgG complexes. *Journal of Immunology*, **103**, 467.

STANWORTH, D.R. & TURNER, M.W. (1973) Immunochemical analysis of immunoglobulins and their subunits. In: *Handbook of Experimental Immunology* (Ed. by D.M.Weir), vol. 1, pp. 10 & 16. Blackwell Scientific Publications, Oxford.

WELBOURN, E., CHAMPION, R.H. & PARISH, W.E. (1976a) Hypersensitivity to bacteria mediating cytotoxic changes in generalised eczema. I. Bacterial culture, skin tests, and immunofluorescent detection of immunoglobulins and bacterial antigens. *British Journal of Dermatology*, **94**, 619.

WELBOURN, E., CHAMPION, R.H. & PARISH, W.E. (1976b). Hypersensitivity to bacteria mediating cytotoxic changes in generalised eczema. III. Four hour Arthus-like skin test responses to bacterial antigens in the absence of specific antibody. *British Journal of Dermatology*, **95**, in press.

British Journal of Dermatology (1976) **95**, 295.

Immunological aspects of psoriasis

II. DISSOCIATED IMPAIRMENT OF THYMUS-DEPENDENT LYMPHOCYTES

J.J.GUILHOU AND J.MEYNADIER
Clinique de Dermatologie et Syphiligraphie, Hôpital Saint-Charles, Montpellier, France

J.CLOT, E.CHARMASSON, M.DARDENNE AND J.BROCHIER
Département d'Immunologie Cellulaire, Hôpital Saint-Eloi, Montpellier, France

Accepted for publication 20 January 1976

SUMMARY

We have investigated lymphocyte subpopulations (six different markers) and T cell functions (mitogen responses and serum thymic factor determination) in twenty patients with psoriasis compared with thirty-five healthy subjects. Normal results were found for B cell markers, but significantly lower numbers of T cells were assessed by E-rosettes and anti HTLA serum. Mitogen responses were normal for PHA but depressed after ConA and PWM stimulation. Lastly, thymic factor levels were significantly higher in psoriasis than in normal subjects of the same age.

These findings could be in favour of a T cell subpopulation defect involved in an auto-immune pathogenesis of psoriasis.

In a general review Epstein (1971) stated: 'The immunologic literature on psoriasis is remarkable not only for its length but for its negativity'. This assertion should now be revised in the light of recent work.

In a preceding paper (Guilhou *et al.*, 1976) we have pointed out some immunoglobulin disturbances: elevated levels of serum IgA (already described by Fraser, Dick & Grichton, 1969; Marghescu & Braun-Falco, 1970; Varelzidis & Theodoridis, 1971), correlated with the extent of involvement and the presence of psoriatic arthropathy; increase of secretory IgA, in agreement with the data of Oon *et al.* (1973); increase of serum IgE in some patients (not previously reported), correlated with serum IgA.

We have also demonstrated the presence of an anti-IgG activity in the serum (45% of the patients), confirming the data from Howell *et al.* (1972) and Florin-Christensen *et al.* (1974). Such anti-IgG factors have been described in psoriatic skin (Krogh & Tonder, 1972) and on peripheral blood lymphocytes by ourselves (Rimbaud *et al.*, 1973).

Cormane, Hunyadi & Hamerlinck (1976) have demonstrated antinuclear antibodies bound to the membranes of circulating lymphocytes.

Recently Beutner *et al.* (1975) confirmed the presence of skin fixed anti-stratum corneum autoantibodies, previously described by Krogh & Tonder (1973).

Studies of cell mediated immunity by means of intradermal tests and DNCB sensitization suggest a slight tendency to depressed sensitization in psoriasis (Depaoli, 1953; Meneghini, Rantuccio & Frugis, 1963; Epstein & Maibach, 1965).

Likewise Hopsu-Havu & Helander (1974) found no consistent abnormality of lymphocyte proliferation induced by phytohaemagglutinin. To our knowledge investigation of lymphocyte markers and function, which is the purpose of the present study, has not been previously reported in the psoriasis literature.

MATERIALS AND METHODS

Patients

Twenty psoriatic patients (16 males and 4 females) aged 18–77 years (mean 52·5 years) were investigated. They had received no immunosuppressive drug for a minimum of 6 months prior to this study. Two of these patients had generalized psoriasis, eight numerous disseminated lesions, six few lesions and four no active lesions. Arthropathy was present in four patients.

Preparation of lymphocyte suspensions

For each patient, 20–30 ml of venous blood were drawn up onto phenol-free heparin. The lymphocytes were obtained by centrifugation on Ficoll–Hypaque density gradient (Böyum, 1968). The cell suspensions contained 85–95% of small lymphocytes and 5–15% of monocytes as judged by peroxidase staining (Preud'Homme & Flandrin, 1974).

T-cell membrane markers

Three markers were used. The first one was the classical E-rosette test according to the method described by Wybran, Chantler & Fudenberg (1973). A second technique was used to identify 'active E-rosettes' as reported by Wybran & Fudenberg (1973). The third marker was evaluated using an heterologous anti-human T-lymphocyte antigen serum (anti-HTLA), prepared by Brochier *et al.* (1976). This antiserum fulfilled all the criteria of specificity defined in a WHO/IARC report (1974).

B-cell membrane markers

Two common markers were used. The B cells bearing surface immunoglobulins (S.Ig) were detected by immunofluorescence staining according to a standardized technique (WHO/IARC Report, 1974). A fluorescein-conjugated anti-total immunoglobulin serum (Melloy Laboratories) was used after specificity tests and ultracentrifugation.

Binding of erythrocytes sensitized with IgM antibody and mouse complement was used to detect lymphocytes carrying complement (C) receptors by EAC-rosettes (Bianco, Patrick & Nussenzweig, 1970).

Detection of Fc-receptor-bearing lymphocytes

We used EA-rosettes according to the method recently described by Brochier, Samarut & Revillard (1975), and using chicken erythrocytes coated by a rabbit anti-chicken IgG serum.

Lymphocyte stimulation by phytomitogens

The micro-method employed was reported elsewhere (Clot, Massip & Mathieu, 1975). The following phytomitogens were used: (a) phytohaemagglutinin (PHA-P) at a concentration of 0·01 ml/ml (b) concanavalin A (conA) at a concentration of 50 μg/ml; pokeweed mitogen (PWM) at a concentration of 0·01 ml/ml. The optimal doses of these mitogens were previously determined by studies of dose–response curves on lymphocyte cultures from normal donors.

Evaluation of thymic hormone in the serum

The procedure used was recently reported by Bach *et al.* (1972). Briefly, fresh sera were ultrafiltered through Amicon membranes to remove the high molecular weight inhibitor present in the serum, and then incubated for 90 min at 37°C with spleen cells from mice thymectomized 10–15 days before. Azathioprine or anti-θ serum was added simultaneously with the serum ultrafiltrate; when thymic hormone was present, more than 50% rosette inhibition was obtained. Control tubes with azathioprine alone or serum ultrafiltrate alone showed no rosette inhibition.

RESULTS

Lymphocyte surface markers

The results obtained are shown in Table 1. They were expressed as the mean percentage of cells ± standard deviation of the mean (s.d.), and as total lymphocyte count per mm^3 ± s.d. Student's '*t*'-test was used for significance of differences in the means. The percentage and the total count of B-lymphocytes were identical in the psoriatic group and in the control group, as evaluated by surface immunoglobulin and EAC-rosettes. In the same way, there was no statistical difference between the mean values of EA-rosettes for the detection of Fc-receptor-bearing lymphocytes.

TABLE 1. Quantitative estimation of lymphocyte markers in twenty patients with psoriasis

	E	'a-E'	HTLA	S.Ig	EAC	EA
Psoriasis	57·9*	25·9	66·1	15·9	15·3	13·1
(20)	±15·4	±12·1	±16	±3·3	±5·5	±7·0
	941·6†	421·9	1063·2	261·1	248·2	205·7
	±332·1	±197·3	±329·2	±107·0	±89·0	±141·6
Controls	70·9*	21·9	79·2	16·2	16·9	12·3
(35)	±6·4	±10·0	±4·3	±3·5	±3·8	±3·4
	1253·3†	387·6	1400·2	285·8	299·7	217·0
	±112·7	±177·1	±76·9	±61·7	±67·2	±61·3
Student's '*t*'-test	$P<0·001$	n.s.	$<0·001$	n.s.	n.s.	n.s.

* Results expressed as mean percentage ± s.d.; † results expressed as total lymphocyte count per mm^3 ± s.d.; n.s. = not significant.

Conversely, the percentage as well as the total count of T-cells, investigated by the E-rosette test and a specific anti-HTLA serum, were significantly depressed in our psoriatic group compared to the controls. The numbers of 'active E-rosettes' were similar in both groups. It was noted that decreased levels of T-cells were found in about one out of two patients. All the tests were repeated twice or three times with quite similar results.

Lymphocyte stimulation by phytomitogens

As indicated in Table 2, only thymidine incorporation induced by ConA and PWM stimulation, and

TABLE 2. ^{3}H-thymidine uptake in *in vitro* lymphocytes cultures in the presence of PHA, ConA and PWM. The results are expressed as dpm × 10^{-3} for 10^6 lymphocytes/ml

Psoriasis (20)	200·1±65·4	104±53·2	76·4±25·0
Controls (35)	216·5±72·4	200±72.7	114·4±55·1
Student's '*t*'-test	*P* n.s.	<0·001	<0·01

expressed as the mean of disintegrations per minute (dpm), significantly differed between the psoriatic group and the control group. The mitogen responses were reduced in psoriasis. However, the PHA response was not significantly modified.

Serum thymic hormone

As previously reported (Rosenthal & Franklin, 1975), the serum level of thymic hormone in man depends on age. It is steady up to the age of 15–20 years, and then declines progressively to become insignificant in most subjects over 40 years.

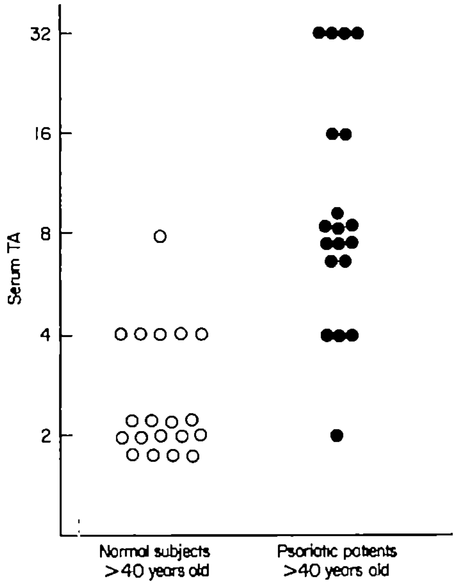


FIGURE 1. Levels of serum thymic factor in psoriasis and in control subjects over 40 years.

Fig. 1 shows the determination of thymic hormone level in the serum of twenty patients with psoriasis compared with thirty-five normal subjects of the same age. Normal or high levels were found in psoriasis, particularly in subjects over 40 years, who showed in 70% of cases a hormone level significantly higher than in normal controls of the same age.

Correlation with clinical features

No correlation was observed between our data and the extent of the lesions, the presence or absence of arthropathy, or the patients' ages.

DISCUSSION

The data reported in this study of patients with psoriasis can be summarized as follows. Thymus-dependent lymphocytes were significantly decreased in psoriatic peripheral blood as evaluated by E-rosettes and anti-HTLA serum. Likewise, the *in vitro* responses to ConA and PWM stimulation were depressed. Conversely, all the other markers ('active-E-rosettes', surface immunoglobulins, EAC- and EA-rosettes) had normal values. Lastly, normal or high thymic factor levels were found in psoriasis. These results were not related to any clinical features; however, this fairly small group of patients may be insufficient to detect such correlations.

A slightly decreased mean percentage of circulating lymphocytes forming E-rosettes has also been found in psoriatic patients recently studied by Cormane, Hunyadi & Hamerlinck (1976) and by Jablonska and co-workers (personal communication). Thus these first findings seem in agreement.

However, many discrepancies appear in our results between the quantitative estimation of lymphocyte markers and T-cell functions. The number of circulating thymus-dependent lymphocytes is clearly decreased, as well as the mitogenic response to ConA and PWM, yet PHA stimulation and thymic hormone levels are not impaired. There have been many reports of the mainly specific stimulation of T-cells by PHA, ConA and PWM (Clot *et al.*, 1975), and of the relation of thymic factor to the presence of the thymus (Bach *et al.*, 1972).

These discrepancies can be explained in several ways. Firstly, thymic factor determination in the serum might be biased by non-thymic products like those produced, for instance, during inflammatory processes. In fact, the serum A related protein (SAA) clearly increases in subjects over 70 years, which rules out the likeness between the two factors (Rosenthal & Franklin, 1975).

Another explanation could be an influence of extrinsic factors on E-rosettes, and on ConA and PWM stimulation. These extrinsic factors could be circulating immune complexes and/or anti-lymphocyte antibodies. Very little such information is known in psoriasis. However, we (Guilhou *et al.*, 1976) and others (Howell *et al.*, 1972; Florin-Christensen *et al.*, 1974) have described the presence of anti-IgG antibodies (rheumatoid factors) in the sera of patients with psoriasis. Such complexes are known to interfere with the assessment of B and T cells in diseases like rheumatoid arthritis and systemic lupus erythematosus. Furthermore, the occurrence of antibodies directed against lymphocyte surface determinants (Winchester *et al.*, 1975), not yet demonstrated in psoriasis, could greatly modify the evaluation of lymphocyte markers and T-cell functions. The absence of a relative increase in B-cell markers, as found in our experiments, would fit with this hypothesis.

Lastly, all these tests explore different T-cell populations and functions, not identically altered in psoriasis. It is possible that the slight decrease of T-cells depends on a subset of thymus-derived lymphocytes, involved, as suppressor cells, in the control of antibody production by B-lymphocytes. The present methods of evaluating lymphocyte markers in peripheral blood are unable to distinguish the subpopulations of helper and suppressor T-cells. However, the likelihood of such populations has been described very recently in human blood (Yu, 1975) and in human thymus (Galili & Schlesinger, 1975).

Such an heterogeneity has also been reported during mitogen stimulation. Recent experiments from Gatien *et al.* (1975) clearly demonstrate that peripheral blood precursor cells from the human thymus respond differently to PHA, ConA and PWM. Other evidence can be advanced for the role of T-suppressor cells in IgA and IgE responses. The production of such immunoglobulins in animals (Van Muiswinkel & Van Soest, 1975) and humans (Geha *et al.*, 1975) requires the co-operation of thymus-dependent lymphocytes. Decreases of suppressor cells enhance antibody production. IgA and IgE levels are significantly increased in psoriasis (Guilhou *et al.*, 1976). Likewise, suppressor T-cells are closely related to auto-antibody production in NZB mice (Steinberg, 1974). The occurrence in psoriasis of auto-antibodies directed against IgG and deoxyribonucleic acid (Cormane *et al.*, 1976) could be due to an impairment of suppressor activity.

Further experiments are now needed to study the subsets of T-lymphocytes involved in psoriasis, and to investigate how they could directly or indirectly be involved in an auto-immune pathogenesis theory of psoriasis.

REFERENCES

Bach, J.F., Dardenne, M., Papiernik, M., Barois, A., Levasseur, P. & Le Brigand, H. (1972) Evidence of a serum factor produced by the human thymus. *Lancet*, **ii**, 1056.

Beutner, E.H., Jablonska, S., Jarsabek-Chorzelska, M., Marciejocuska, E., Genoweda, R. & Chorzelski, T. (1975) Studies in immunodermatology (VI). Immunofluorescence studies of auto-antibodies to the stratum corneum and of *in vivo* fixed IgG in stratum corneum of psoriatic lesions. *International Archives of Allergy and Applied Immunology*, **48**, 301.

Bianco, C., Patrick, R. & Nussenzweig, V. (1970) A population of lymphocytes bearing a membrane receptor for antigen-antibody-complement complexes. I. Separation and characterization. *Journal of Experimental Medicine*, **132**, 702.

Böyum, A. (1968) Separation of leukocytes from blood and bone marrow. *Scandinavian Journal of Clinical and Laboratory Investigations*, **21**, suppl. 97.

Brochier, J., Abou Hamed, Y.A., Gueho, J.P. & Revillard, J.P. (1976) Study of human T and B lymphocytes with heterologous antisera. I. Preparation, specificity and properties of antisera. *Immunology* (in press).

Brochier, J., Samarut, C. & Revillard, J.P. (1975) A rosette technique for identification of human mononuclear cells bearing Fc receptors. *Biomedicine Express*, **23**, 206.

Clot, J., Massip, H. & Mathieu, O. (1975) *In vitro* studies on human B and T cell purified populations. Stimulation by mitogens and allogeneic cells, and quantitative binding of phytomitogens. *Immunology*, **29**, 445.

Cormane, R.H., Hunyadi, J. & Hamerlinck, F. (1976) Les mécanismes immunologiques du psoriasis. *Annales de Dermatologie et de Syphiligraphie* (in press).

Depaoli, M. (1953) Uber die toxische Reaktion und die Sensibilisierung gegen 2–4 Dinitrochloroben zol bei Psoriassis-Kranken. *Hautarzt*, **4**, 319.

Epstein, W.L. (1971) Immunologic factors in psoriasis. (Ed. by E.M.Farber and A.J.Cox), p. 297. Stanford University Press, Stanford.

Epstein, W.L. & Maibach, H.L. (1965) Immunologic competence of patients with psoriasis receiving cytotoxic drug therapy. *Archives of Dermatology*, **91**, 599.

Florin-Christensen, A., Maldonato Cocco, J.A., Arana, R., Porrini, A., Mom, A. & Garcia Morteo, O. (1974) Antigammaglobulin factors in psoriasis. *Dermatologica*, **149**, 220.

Fraser, N.G., Dick, H.M. & Grichton, W.B. (1969) Immunoglobulins in dermatitis herpetiformis and various other skin diseases. *British Journal of Dermatology*, **81**, 89.

Galili, U. & Schlesinger, M. (1975) Subpopulations of human thymus cells differing in their capacity to form stable E-rosettes and in their immunologic reactivity. *Journal of Immunology*, **115**, 827.

Gatien, J.G., Schneeberger, E.E., Parkman, R. & Merler, E. (1975) Isolation on discontinuous gradients of bovine albumin of a subpopulation of human lymphocytes exhibiting precursor characteristics. *European Journal of Immunology*, **5**, 306.

Geha, R.S., Colten, H.R., Schneeberger, E. & Merler, E. (1975) Co-operation between human thymus-derived and bone marrow-derived lymphocytes in the antibody response to ragweed antigen E *in vitro*. *Journal of Clinical Investigation*, **56**, 386.

Guilhou, J.J., Clot, J., Meynadier J. & Lapinski H. (1976) Immunological aspects of psoriasis. I. Immunoglobulins and anti-IgG factors. *British Journal of Dermatology*, **94**, 501.

Hopsu-Havu, U.K. & Helander, I. (1974) Cell mediated auto-hypersensitivity in psoriasis. *Acta dermato-venereologica*, **54**, 333.

Howell, F.A., Chamberlain, M.A., Perry, R.A., Torrigiani, G. & Roitt, I.M. (1972) IgG antiglobulin levels in patients with psoriatic arthropathy, ankylosing spondylitis and gout. *Annals of the Rheumatic Diseases*, **31**, 129.

Krogh, H.K., Tonder, O. (1972) Immunoglobulins and anti-immunoglobulin factors in psoriatic lesions. *Clinical and Experimental Immunology*, **10**, 623.

Krogh, H.K. & Tonder, O. (1973) Antibodies in psoriatic scales. *Scandinavian Journal of Immunology*, **2**, 45.

MARGHESCU, S. & BRAUN-FALCO, O. (1970) Quantitatives Verhalten von Immunoglobulinen und Komplement bei Psoriasis. *Archiv für Klinische und Experimentelle Dermatologie*, **238**, 417.

MENEGHINI, C.L., RANTUCCIO, F. & FRUGIS, E. (1963) Sensibilisation eczemateuse experimentale au DNCB chez l'homme. *Dermatologica*, **127**, 298.

OON, C.H., GOODWIN, P.G., KIND, P.R.N., SEAH, P.P. & FRY, L. (1973) Salivary IgA in patients with psoriasis and dermatitis herpetiformis. *Acta dermato-venereologica*, **53**, 340.

PREUD'HOMME, J.L. & FLANDRIN, G. (1974) Identification by peroxidase staining of monocytes in surface immunoglobulin tests. *Journal of Immunology*, **113**, 1650.

RIMBAUD, P., MEYNADIER, J., GUILHOU, J.J. & CLOT, J. (1973) Anti-IgG activity on peripheral blood lymphocytes in psoriasis. *Archives of Dermatology*, **108**, 371.

ROSENTHAL, C.J. & FRANKLIN, E.C. (1975) Age-associated changes of an amyloid related serum component. *Journal of Clinical Investigation* (in press).

STEINBERG, A.D. (1974) Pathogenesis of autoimmunity in New Zealand mice. V. Loss of thymic suppressor function. *Arthritis and Rheumatism*, **17**, 11.

VAN MUISWINKEL, W.B. & VAN SOEST, P.L. (1975) Thymus dependence of the IgA response to sheep erythrocytes. *Immunology*, **28**, 287.

VARELZIDIS, A. & THEODORIS, A. (1971) Serum immunoglobulins in psoriasis before and after ultraviolet light therapy. *British Journal of Dermatology*, **105**, 695.

WINCHESTER, R.J., WINFIELD, J.B., SHU MAN FU, WERNET, P. & KUNKEL, H.G. (1975) Studies on antilymphocyte antibodies in patients with rheumatoid arthritis and systemic lupus erythematosus. Immunological aspects of rheumatoid arthritis. *Rheumatology* (ed. by Karger, Basel), **6**, 209.

WHO/IARC REPORT (1974) Identification, enumeration and isolation of B and T lymphocytes from human peripheral blood. *Scandinavian Journal of Immunology*, **3**, 521.

WYBRAN, J., CHANTLER, S. & FUDENBERG, H.H. (1973) Isolation of normal T cells in chronic lymphatic leukaemia. *Lancet*, **i**, 126.

WYBRAN, J. & FUDENBERG, H.H. (1973) Thymus-derived rosette-forming cells in various human disease states: cancer, lymphoma, bacterial and viral infections, and other diseases. *Journal of Clinical Investigation*, **52**, 1026.

YU, D.T.Y. (1975) Human lymphocyte subpopulations: early and late rosettes. *Journal of Immunology*, **115**, 91.

British Journal of Dermatology (1976) **95**, 303.

Alternative pathway complement activation: a possible mechanism inducing skin lesions in benign gonococcal sepsis

REINER SCHERER AND OTTO BRAUN-FALCO

Department of Dermatology, University of Munich, West Germany

Accepted for publication 5 January 1976

SUMMARY

In order to elucidate the pathogenesis of the skin lesions in 'benign gonococcal sepsis' direct immunofluorescence of an early macular lesion and routine histopathology of a mature papulopustular lesion in a patient with septic gonococcal dermatitis have been performed. Histopathology of the mature skin lesion revealed a pattern of 'allergic vasculitis'. Direct immunofluorescence showed exclusively deposits of C3 around and within the capillaries and in the basement membrane zone. No specific IgG, IgM, IgA or C4 deposits could be demonstrated. This, together with serological findings and reports from the literature, suggests an important pathogenetic function for complement, activated through the alternative pathway by means of gonococcal endotoxic lipopolysaccharide, in the pathogenesis of the skin lesions in benign gonococcal sepsis.

'Benign gonococcal sepsis' or, as it is also referred to in the literature, 'gonococcal septicaemia', 'septic gonococcal dermatitis', or 'generalized gonorrhoea' is characterized clinically by the triad of fever, arthralgia and typical cutaneous lesions. The striking similarity between the histopathological findings from these skin lesions and those in allergic vasculitis has been noted by ourselves (Braun-Falco, 1973) as well as by others (Shapiro, Teisch & Braunstein, 1973; Schröter, Bersch & Osswald, 1975). To date no entirely satisfactory explanation for this phenomenon has been proposed. We would like to report some immunofluorescent findings which lead us to suggest a new pathogenetic mechanism for the skin lesions occurring in gonococcaemia.

CASE REPORT

A 26-year-old female tourist presented as an outpatient for treatment of 'fever blisters' following a 'high fever' 48 hours before. On examination papulopustular and haemorrhagic skin lesions predominantly located on the upper and lower limbs were detected. She complained also of joint pains

---

Correspondence: Professor O. Braun-Falco, Department of Dermatology, University of Munich, Frauenlobstrasse 9, D-8000 München 2, West Germany.

most prominently in her right knee, which was found to be slightly swollen. Symptoms of pelvic inflammatory disease were denied. On pelvic examination no leucorrhoea or other obvious pathological findings were noted, but intracellular Gram-negative diplococci were demonstrated in a cervical smear and *Neisseria gonorrhoeae* were cultured from cervical mucus. Microscopic examination of urethral and cervical secretions revealed a Trichomonas infection as well.

The patient was admitted to hospital for treatment. She was in good general condition and was not febrile (36·6°C). The WBC was 6100/mm^3 with 65% neutrophils, 30% lymphocytes and 3% eosinophils. Hb was 13·6 g%. Urinalysis was normal. ESR was increased to 48 mm/h. Serum uric acid, urea, creatinine, SGOT, SGPT and total bilirubin were within normal limits. The results of serum protein electrophoresis were: albumin 54·5%, α-1-globulins 6%, α-2-globulins 13%, β-globulins 10·5%, γ-globulins 16% with a total serum protein concentration of 7·0 g/100 ml. Results of quantitative determination of specific serum proteins by radial immunodiffusion (Partigen-plates, Behringwerke, Marburg) are summarized in Table 1.

TABLE 1. Patient's serum concentration of immunoglobulins and complement components C3 and C4 as compared to mean values and normal concentration range in healthy adults

Serum protein	Serum concentration in the patient (mg/100 ml)	Mean serum concentration in healthy adults (mg/100 ml)	Normal concentration range in healthy adults (mg/100 ml)
IgG	1340	1250	800–1800
IgA	200	210	90–450
IgM	130	160	70–280
C3 (β_1C/β_1A)	85	110	80–140
C4 (β_1E)	45·4	30	20–50

The gonococcal complement-fixation test was performed on the day of admission and again after 8 days of treatment with intravenous infusions of 10,000,000 penicillin G daily; both were negative. Complement-fixation tests for Coxsackie virus type A_2,A_7,A_9 and B_3 were also negative. Only the urethral and cervical cultures were positive for *Neisseria gonorrhoeae* whereas cultures from several skin lesions and aerobic and anaerobic blood cultures were sterile.

The patient presented skin lesions at different stages of development: pinpoint erythematous macules evolved into petechiae, maculopapules, papulopustules and haemorrhagic pustules 2–4 mm in diameter that were surrounded by an intense erythematous halo.

Two biopsies were taken: (a) An erythematous, slightly haemorrhagic macule, which represented the earliest visible stage, was excised and immediately placed into liquid nitrogen for processing by direct immunofluorescence. (b) An isolated small papulopustular lesion, which represented a mature stage, was excised and processed for routine histopathological examination by fixation in 10% formalin, embedding in paraffin, and finally haematoxylin–eosin staining.

The excised macule was studied for the presence of deposited immunoglobulins and complement components with fluorescein isothiocyanate (FITC) conjugated monospecific antibodies to human IgG, IgA, IgM and C3 (β_1C/β_1A) (antisera from Hyland-Travenol, Munich); demonstration of tissue-bound C4 was attempted with rabbit anti-C4 using FITC labelled goat antirabbit serum as a second antibody. Tissue sections from normal skin excised at surgery served as negative controls. Positive controls were skin biopsies from patients with bullous pemphigoid and dermatitis herpetiformis which were both obtained by chance on the same day. The positive controls showed the characteristic immunofluorescence patterns seen in these diseases (Van Joost, 1975), i.e. linear deposits of IgG, C3 and C4 in the basement membrane zone with bullous pemphigoid, and granular IgA deposits in the dermal papillae with dermatitis herpetiformis. In striking contrast, the early macular lesion in our patient with gonococcal septicaemia showed no specific immunofluorescence with antisera to IgG, IgA, IgM and C4, but did show deposition of C3 within the walls of arterioles (Fig. 1a) and virtually all capillaries; staining of both ascending and descending loops of capillaries in dermal papillae (Fig. 1b) was demonstrated. In addition granular C3 deposition was seen along the basement membrane zone (Fig. 1a, b). These findings suggest *activation and tissue fixation of C3 in the absence of demonstrable immune complexes* at this early stage of skin involvement in septic gonococcal dermatitis. Identical immunofluorescent findings were seen in another patient with characteristic clinical symptoms of benign gonococcal sepsis. Unfortunately cultures for *N. gonorrhoea* were negative in this patient due to antibiotic treatment prior to hospital admission.

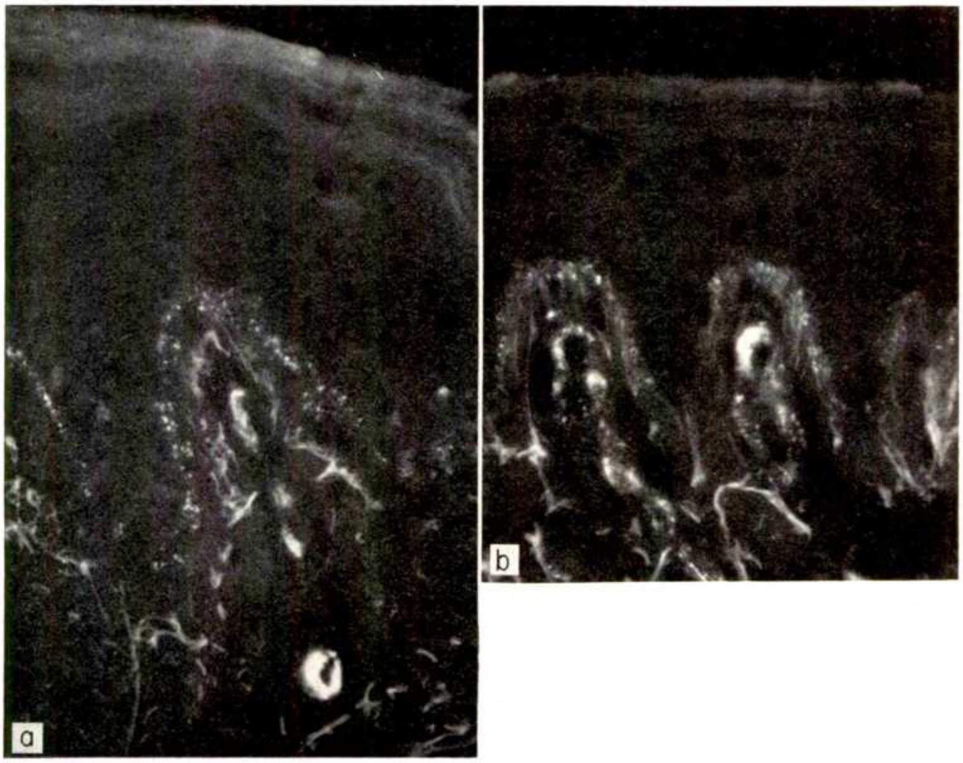


FIGURE 1. (a and b) 'Benign gonococcal sepsis': sections from the early macular skin lesion. C3 deposits are in arterioles, in ascending and descending capillary loops and near the basement membrane zone. Conjugate: Goat antihuman C3 (β_1C/β_1A), weight ratio fluorescein/protein: 8·5 μg/mg, molar ratio specific antibody protein/protein: 0·17, molar ratio specific antibody protein/fluorescein: 0·048, specific antibody concentration: 1·9 mg/ml, dilution used: 1:64. (Original magnification × 100.)

The H & E-stained specimen from the papulopustular type of skin lesion revealed a histopathological pattern identical to the one described as 'allergic vasculitis' (Ruiter, 1964). The most prominent features were (Fig. 2a, b):

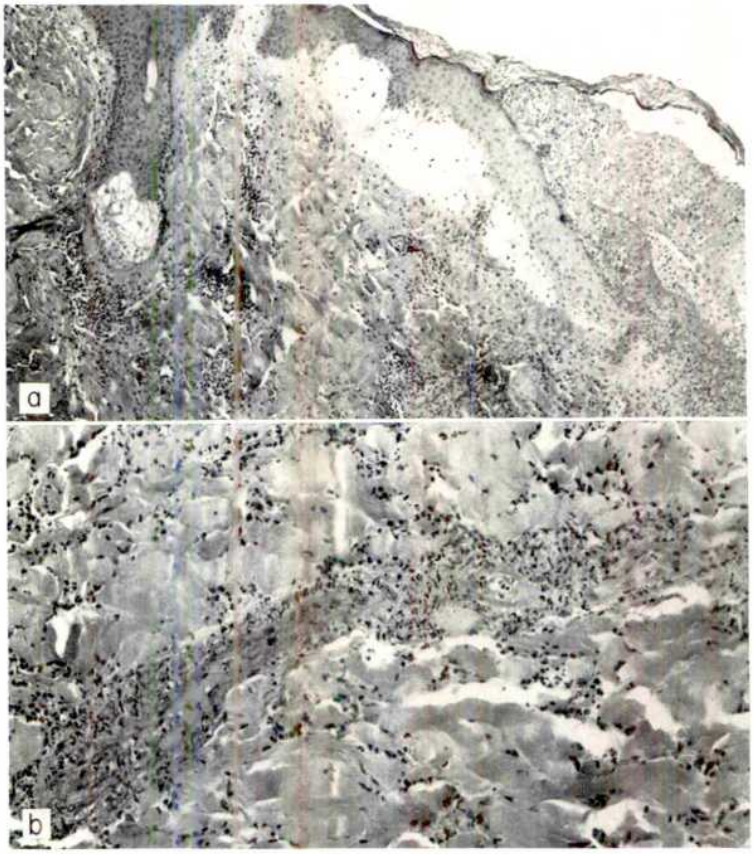


FIGURE 2. 'Benig ngonococcal sepsis': H & E-stained sections from a mature papulopustular skin lesion. (a) shows perivascular infiltration of polymorphonuclear neutrophils, oedema and the presence of a subepidermal pustule (× 80). (b) shows a thrombosed blood vessel and perivascular infiltration by polymorphonuclear neutrophils (× 120).

Vascular damage with endothelial swelling, fibrinoid degeneration of blood vessel walls, infiltration with polymorphonuclear leukocytes and vascular occlusion due to microthrombi.

Perivascular infiltrate dominated by polymorphonuclear neutrophils, leukocytoclasis, and extravasation of red blood cells in the inflammatory infiltrate.

Oedema of epidermis and subpapillary layer.

Presence of a subepidermal pustule filled with necrotic material and with partial destruction of the overlying epidermis.

DISCUSSION

The clinical symptoms as well as the usual histopathological findings presented in this case report are typical and well in agreement with the characteristic features of benign gonococcal sepsis as described in previous reports (Shapiro *et al.*, 1973; Schröter *et al.*, 1975):

(1) A female patient—85% of the patients with gonococcaemia are women (Schröter *et al.*, 1975)—came to the clinic without any suspicion of venereal disease, because genital symptoms were absent.

(2) She presented with typical skin lesions, arthralgia and a history of fever. All the symptoms disappeared within a few days after high dose penicillin therapy was begun.

(3) Histopathological findings from a mature papulopustular lesion closely resembled the pattern seen in allergic vasculitis.

The further investigations reported here were directed towards elucidation of the mechanism producing this specific type of skin lesion in gonococcal sepsis.

Three theories have been proposed to explain the pathogenesis of the histopathological alterations in septic gonococcal dermatitis:

(1) Embolization to the cutaneous blood vessels by gonococcal organisms (Danielsson & Michaëlsson, 1966; Kahn & Danielsson, 1969).

(2) A hypersensitivity mechanism related to the 'Arthus phenomenon' (Wiedmann, 1934).

(3) The 'Shwartzman phenomenon' (Ackerman *et al.*, 1965; Shapiro *et al.*, 1973; Schröter *et al.*, 1975).

Just as in our patient, attempts to culture *N. gonorrhoea* from skin lesions failed in 43 of 44 reported cases (Kahn & Danielsson, 1969). Blood cultures have been successful only in 20% of the examined cases (Schröter *et al.*, 1975). These results make the theory of microbial embolization doubtful, although they may indicate that this special organism is rapidly destroyed or inactivated by humoral defence mechanisms. Using FITC-labelled antigonococcus globulin, gonococcal antigens not detected by conventional staining methods have been demonstrated within skin lesions and around nearby blood vessels by direct immunofluorescence (Kahn & Danielsson, 1969). Our own immunofluorescent findings from an early skin lesion suggest activation and subsequent tissue fixation of complement C3 in and around capillaries and at the dermo-epidermal junction in the absence of antigen–antibody complexes.

Endotoxin is a lipopolysaccharide derived from the outer membrane of Gram-negative bacteria and it is present also in *N. gonorrhoea* (Boor & Miller, 1944); it is known to activate C3 by the alternative pathway without the participation of immune complexes and thus bypasses the early classical C1, C4 and C2 components (Gewurz, 1972). Activation of the complement system by the properdin pathway in patients with Gram-negative sepsis has been reported (Fearon *et al.*, 1974). These patients had a marked decrease in C3 serum concentration, while the C4 concentration was normal. In our patient (see Table 1) C4 concentration was above the mean normal value, while the C3 concentration was near the lower limit of normal values.

Recently, circulating immune complexes in a patient with prolonged gonococcal septicaemia have been described (Danielsson, Norberg & Svanbom, 1975), but this might be ascribed to the long duration of the disease in that patient. Furthermore, that patient was splenectomized and thus deprived of part of his reticulo-endothelial system. It has been shown that a high antibody titre to gonococcal antigen as determined by positive complement fixation tests can only be expected 2–3 weeks after diagnosis and subsequent treatment (Danielsson *et al.*, 1972). In the acute phase of gonococcal sepsis only 35–40% of complement fixation tests yield positive results. Thus the negative results from complement fixation tests in our patient confirmed the absence of a marked antibody specific to gonococcal antigen.

If one assumes that nonspecific complement activation occurred in our patient due to the presence of endotoxic bacterial lipopolysaccharide, then the pathogenesis of the histopathological alterations within the skin of the patient can be explained as summarized in Figs 3 and 4: complement activation

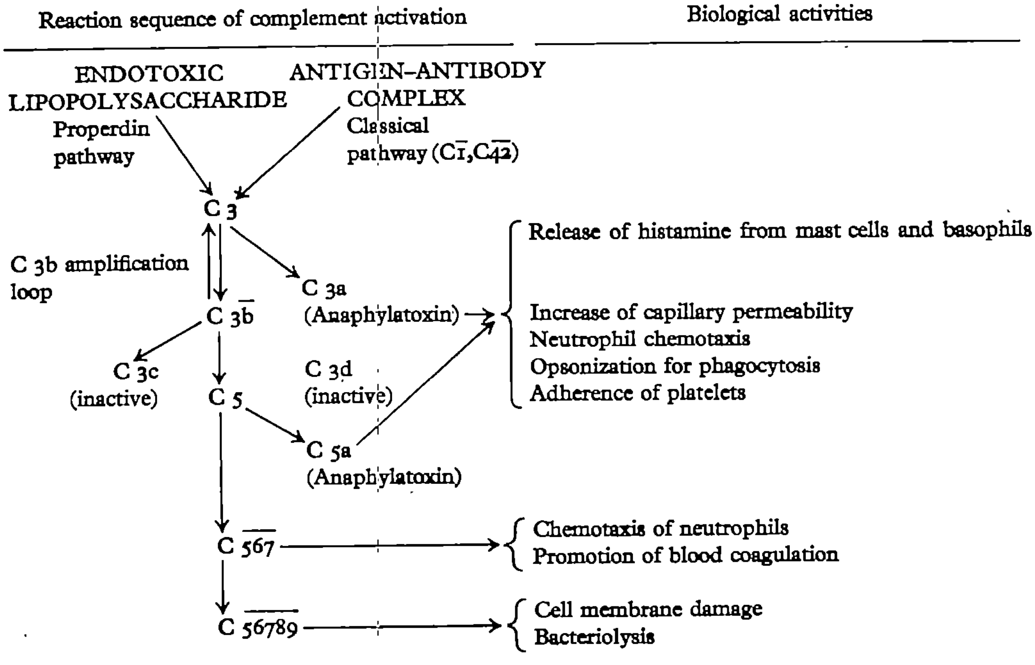

Reaction sequence of complement activation
Biological activities
ENDOTOXIC LIPOPOLYSACCHARIDE
ANTIGEN-ANTIBODY COMPLEX
Properdin pathway
Classical pathway (C1,C42)
C 3
C 3b amplification loop
C 3b
C 3a (Anaphylatoxin)
C 3c (inactive)
C 3d (inactive)
C 5
C 5a (Anaphylatoxin)
Release of histamine from mast cells and basophils
Increase of capillary permeability
Neutrophil chemotaxis
Opsonization for phagocytosis
Adherence of platelets
C 567
Chemotaxis of neutrophils
Promotion of blood coagulation
C 56789
Cell membrane damage
Bacteriolysis

FIGURE 3. Mechanism and biological consequences of complement activation.

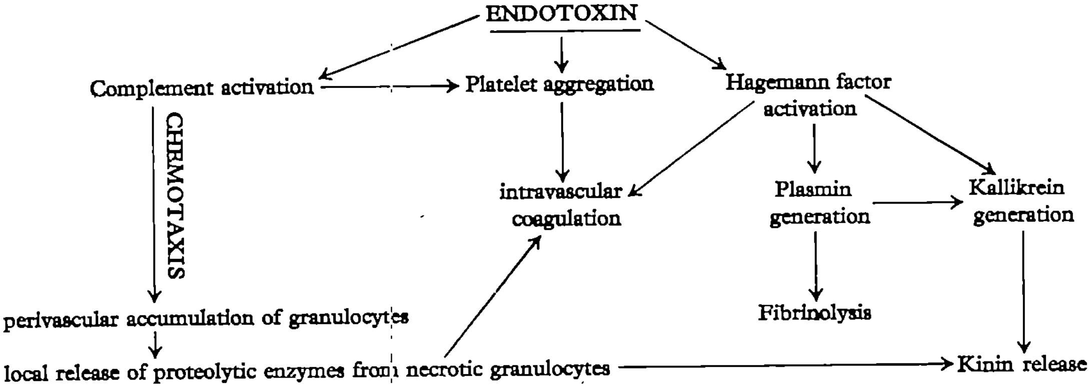

ENDOTOXIN
Complement activation
Platelet aggregation
Hagemann factor activation
CHEMOTAXIS
intravascular coagulation
Plasmin generation
Kallikrein generation
Fibrinolysis
perivascular accumulation of granulocytes
local release of proteolytic enzymes from necrotic granulocytes
Kinin release

FIGURE 4. Interaction of complement, clotting, fibrinolytic and kinin releasing systems during inflammatory reaction triggered by bacterial endotoxin.

generates the anaphylatoxins C3a and C5a (Fig. 3) which together with the released kinins (Fig. 4) increase vascular permeability with subsequent leakage of plasma to produce interstitial oedema and the extravasation of blood cells. Also perivascular attraction of polymorphonuclear leukocytes is mediated by the chemotactic properties of activated complement components (Fig. 3) (Cormane, 1974). The granular C3 deposition in the basement membrane zone (Fig. 1a) might thus represent the initiating event, which eventually leads to subepidermal pustule formation (Fig. 2a). Intravascular coagulation (Fig. 2b) although reinforced by activated complement is probably more efficiently promoted by the other involved systems outlined in Fig. 4. In addition, from Fig. 4 it can be seen that the multiple interactions of complement, clotting, fibrinolytic and kinin generating systems result in a potent amplification system, so that any degree of inflammatory response ranging from local skin lesions up to a fatal shock syndrome can be produced. Fatal sepsis in gonococcaemia is extremely rare due to the efficiency of antibiotics and the relatively low virulence of *N. gonorrhoea*. In meningococcal sepsis, where an almost identical endotoxic lipopolysaccharide is present (Boor & Miller, 1944) and similar skin lesions occur, the risk of amplification of the inflammatory response up to a fatal outcome is considerably greater. The high potency of meningococcal lipopolysaccharide in producing purpuric skin lesions has recently been investigated in detail (Davis & Arnold, 1974).

For positive proof of the suggested alternative pathway complement activation in gonococcal sepsis demonstration of deposits of properdin and of C3 proactivator in the early skin lesions have to be performed in future investigations. We view the results and the rather broad conclusions drawn from this single case report as a starting point for a re-evaluation of the mechanisms involved in cases of so-called 'superficial allergic vasculitis'. Their mere labelling as examples of the 'Arthus phenomenon' or 'Shwartzman phenomenon' although convenient in clinical practice, is not sufficient in explaining the essential mechanisms involved.

In addition we view the reported findings in this case as a typical example of the accumulating knowledge about the importance of nonspecifically triggered and amplified pathophysiological processes, which have formerly been attributed to presumed specific immunological reactions.

ACKNOWLEDGMENT

The excellent technical assistance of Miss Annette Hess is gratefully acknowledged.

REFERENCES

Ackerman, A.B., Miller, R.C. & Shapiro, L. (1965) Gonococcemia and its cutaneous manifestations. *Archives of Dermatology*, **91**, 227.

Boor, A.K. & Miller, C.P. (1944) A carbohydrate–lipid fraction of gonococcus and meningococcus. *Journal of Infectious Diseases*, **75**, 47.

Braun-Falco, O. (1973) In: *Fortschritte der prakt. Dermatologie und venerologie*, Vol. 7, p. 320. Springer, Berlin.

Cormane, R.H. (1974) Neutrophilen-vermittelte Immunreaktionen. *Der Hautarzt*, **25**, 254.

Danielsson, D. & Michaëlsson, G. (1966) The gonococcal dermatitis syndrome. *Acta dermato-venerologica*, **46**, 261.

Danielsson, D., Thyressen, N., Falk, V. & Barr, J. (1972) Serologic investigation of the immune response in various types of gonococcal infection. *Acta dermato-venerologica*, **52**, 467.

Danielsson, D., Norberg, R. & Svanbom, M. (1975) Circulating immune-complexes in a patient with prolonged gonococcal septicemia. *Acta dermato-venerologica*, **55**, 301.

Davis, C.E. & Arnold, K. (1974) Role of meningococcal endotoxin in meningococcal purpura. *Journal of Experimental Medicine*, **140**, 159.

Fearon, D.T., Ruddy, S., McCabe, W.R. & Schur, P.H. (1974) Activation of the complement system by the properdin pathway in patients with Gram-negative bacteremia. *Journal of Clinical Investigation*, **53**, 23a.

Gewurz, H. (1972) Alternate pathways to activation of the complement system. In: *Biological Activities of Complement*, pp. 56–88. Karger, Basel.

Kahn, G. & Danielsson, D. (1969) Septic gonococcal dermatitis. *Archives of Dermatology*, **99**, 421.

Ruiter, M. (1964) Arteriolitis (vasculitis) 'allergica' cutis (superficialis). A new dermatological concept. *Dermatologica*, **129**, 217.

Schröter, R., Bersch, A. & Osswald, F. (1975) Beitrag zur kenntnis der sogenannten 'benignen Gonokokkensepsis'. *Zeitschrift für Haut- und Geschlechtskrankheiten*, **50**, 637.

Shapiro, L., Teisch, T.J.A. & Braunstein, M.H. (1973) Dermato-histopathology of chronic gonococcal sepsis. *Archives of Dermatology*, **107**, 403.

Van Joost, Th. (1975) Circulating autoantibodies in skin diseases a survey and comparison of immunofluorescence (IF) studies. *International Journal of Dermatology*, **14**, 379.

Wiedmann, A. (1934) Die geweblichen Veränderungen beim hämorrhagischen Gonokokkenexanthem mit besonderer Berücksichtigung der Gefäßveränderungen. *Dermatologische Wochenschrift*, **98**, 541.

British Journal of Dermatology (1976) 95, 311.

The effect of long term tetracycline treatment for acne vulgaris on the occurrence of R factors in the intestinal flora of man

M.V.VALTONEN, V.V.VALTONEN, O.P.SALO AND P.H.MÄKELÄ

Central Public Health Laboratory, Helsinki and the Department of Dermatology,
University Central Hospital, Helsinki

Accepted for publication 22 January 1976

SUMMARY

R factors are known to be the most important mechanism of antimicrobial resistance of intestinal flora. Short courses with therapeutic doses (1000 mg/day) of tetracycline select for strains containing transferable resistance factors to more than one antimicrobial agent. In this report we show that long term treatment with very low doses (100 mg/day) of tetracycline for acne vulgaris has an equally strong effect favouring establishment of resistant strains and R factors in the intestinal flora of patients.

Bacteria are hosts to resistance transfer factors (RTF) which form a group of plasmids, extrachromosomal genetic particles. They can carry a variety of genes called resistance (R) determinants or R factors which confer resistance to various antimicrobials on their host bacteria. RTF are able to transfer their genetic information (including the R factors) by conjugation to other bacteria.

Transferable R factors now form the major way by which enteric bacteria become resistant to antimicrobial agents (Anderson, 1968). Oral chemotherapy is believed to be an important selective influence favouring the establishment of resistant strains, a high proportion of which carry R factors. Datta *et al.* (1971) showed that oral tetracycline at the therapeutic level of 1000 mg/day was a more efficient selective agent than such commonly used antimicrobials as ampicillin and sulphonamides. The effects on the faecal flora had, however, disappeared within 5 weeks after treatment. In some other studies, R factor-containing resistant strains could be shown to survive in the intestinal flora for much longer periods (Jonsson, 1974; Hartley & Richmond, 1975). Much lower doses of tetracycline are used in the treatment for acne vulgaris, but the courses are long (Leading article, B.M.J., 1973; Smith & Mortimer, 1967). We thought it of interest to see what effect low doses of tetracycline have on the faecal flora of patients.

PATIENTS AND METHODS

Patients. Thirteen patients (9 females, 4 males; 15–30 years of age) were treated for 2 months with

Correspondence: Dr P.H.Mäkelä, Central Public Health Laboratory, Mannerheimintie 166, SF-00280 Helsinki 28, Finland.

tetracycline for acne vulgaris. Before including them in this study it was ascertained that they had no history of antimicrobial treatment for at least 1 year.

Antibiotic treatment. The patients were given 800 mg of tetracycline a day orally for the first 5 days. After that the oral dose was 100 mg of tetracycline once a day for the remaining 55 days of the treatment.

Faecal samples. Faecal samples were collected from each patient 7 and 0 days before the treatment, at days 30 and 60 of the treatment and 1 year after the treatment.

Method 1. Each faecal sample was streaked on a Drigalski-Conradi (nutrient agar 1000·0 ml, lactose sol. 30·0 ml, bromcresol purple 1·9 ml, pH 7·1) agar plate from which coliform colonies were identified on the basis of colony morphology and yellow colour (lactose fermentation). Twenty colonies were picked at random and confirmed as *E. coli* by standard biochemical tests, and tested for sensitivity to tetracycline (T), sulphonamide (Su), ampicillin (A) and streptomycin (S) with standard discs purchased from Biodisk (Ericson, Högman & Wichman, 1954).

One tetracycline resistant and all multiresistant (resistant to two or more drugs tested) strains of each sample were tested as donors for transfer of resistance to a recipient *E. coli* K 12 strain resistant to sodium azide and requiring methionine for growth. Log. phase broth cultures of donor and recipient were mixed (1:10), incubated for 2 h at 37°C, and spread in 10-fold dilutions on different selective plates. These contained sodium azide (to prevent the growth of the donor) and the antimicrobial to be tested to inhibit the growth of the recipient; on these plates only recipient bacteria, which had acquired an R factor, could grow. One colony from each plate was purified by single colony isolation on nutrient agar and tested for antimicrobial sensitivity. It was also tested for methionine requirement as an additional marker of the recipient.

Method 2. Each faeces sample was also plated in 10-fold dilutions on nutrient agar plates containing tetracycline 1 mg/100 ml and the number of colonies estimated. Two colonies with coliform morphology were picked up at random and restreaked on nutrient agar for single colony isolation; if they were coliforms they were also tested for antimicrobial sensitivity. One tetracycline resistant strain and all different multiresistant strains of each sample were tested for transferable resistance as above.

RESULTS

Pretreatment samples

Almost all the pretreatment samples contained only sensitive strains of coliforms. Transferable R factors were demonstrated in the specimens of one patient only.

The two pretreatment samples gave very similar results (Table 1). One patient provided no pretreatment samples and two patients provided only one. In eight patients (67%) all the strains tested were fully sensitive against T, A, S and Su.

Two patients (Nos 6 and 2) had T and two had A resistant strains, in both cases as a minority population (10% or less of colonies). One of the thirteen patients (No. 4) had multiresistant bacteria (T, A, S, Su) as the predominant flora of both the samples (39 of 40 colonies tested); another (No. 13) had a T, A, S resistant strain as a minority fraction (2 of 40 colonies tested).

Patient No. 13 who had a T, A, S resistant strain as a minority fraction was the only one of the 12 patients (8%) carrying transferable drug resistance in the pretreatment samples. Transfer of resistance to streptomycin was demonstrated in the first sample, and against sulphonamide in the second specimen.

TABLE 1. Number of colonies resistant to tetracycline (T), resistant to any other drug (alone or in combination with T), and multiresistant among 20 colonies tested from each sample

Patient no.	Pretreatment*			Treatment†			Post-treatment‡		
	T	Other	Multi	T	Other	Multi	T	Other	Multi
1	— —	— —	— —	20–20	— —	— —	20	—	—
2	12–1	— —	— —	20–20	0–20	0–20	9§	9§	9§
3	— —	— —	— —	17–20	3–1	2–1	—	—	—
4	20–19	20–19	20–19	0–20	— —	— —	NS¶	NS	NS
5	— NS	— NS	— NS	NS–4	NS–19	NS–3	—	20	—
6	— NS	— NS	— NS	20–20	— —	— —	NS	NS	NS
7	— —	— —	— —	20–0	20–18	20–0	—	—	—
8	— —	— —	— —	20–0	0–19	— —	NS	NS	NS
9	— —	— —	— —	20–20	20–19	20–19	NS	NS	NS
10	NS NS	NS NS	NS NS	— —	4–0	— —	1	1	1
11	— —	— —	— —	20–20	— —	— —	NS	NS	NS
12	— —	— —	— —	17–20	17–20	17–20	NS	NS	NS
13	1–3	8–6	1–1	20–20	7–20	20–20	NS	NS	NS

* First value for the sample 7 days, second value 0 days before treatment; † first value for the sample 30 days, second value 60 days after start of treatment; ‡ 12 months after end of treatment; § 9 colonies of coliforms tested; ¶ NS = no sample.

The T, A, S, Su resistant coliforms of patient No. 4, who had this multiresistant strain as the majority in her faeces, did not transfer resistance to the test strain of *E. coli*.

Treatment samples

Almost all the treatment samples contained only tetracycline resistant strains of coliforms. These were in most cases also resistant to other drugs, and could transfer R factors to *E. coli* K 12.

The resistance patterns of the predominant flora in the treatment samples were changed very drastically compared to the pretreatment samples. The number of colonies on selective plates from samples at days 30 and 60 of the treatment increased on average 1000-fold compared with the pretreatment samples (Fig. 1).

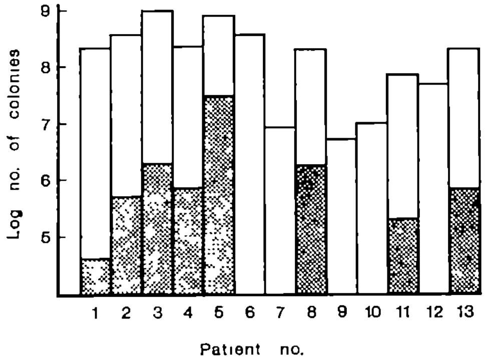


FIGURE 1. Log no. of coliform colonies on selective medium containing tetracycline 1 mg/100 ml. Hatched column, pretreatment; open column, treatment.

Only one patient (No. 10) had a fully sensitive strain as the predominant flora. As a minority he had a sulphonamide resistant strain (4 of 40 colonies tested). From patient No. 5 we had only one treatment sample (at day 60) in which the predominant flora was sulphonamide resistant (16 of 20 colonies tested); of the other 4 colonies one was T resistant, one T, A, Su and two T, S, Su resistant. These resistance patterns were transferable.

All the other patients (11 of 13 patients, 85%) had tetracycline resistant strains, as a majority of the faecal flora (Table 1). In six patients the predominant flora (196 of 240 colonies tested) was tetracycline resistant but sensitive to other drugs, although in the specimen from patient No. 8 at day 60 a strain resistant to sulphonamide alone was predominating. In two cases these strains transferred resistance to T. In one of these patients some multiresistant strains (T, Su, A) were found as a minority.

In five patients the predominant flora was multiresistant: T, A, S, Su in 120 of 200 colonies, with other resistance combinations as a minority (Table 1). All these patients were RTF positive (Table 2) and two of them (Nos 12 and 13) were able to transfer resistance to seven drugs (T, A, S, Su, neomycin = N, kanamycin = K, carbenicillin = Ca). Multiresistance was more often transferable than single resistance (Table 3).

Post-treatment samples

Antimicrobial resistance and transferable R factors could be demonstrated in half of the specimens. Post-treatment samples were obtained from only six patients (Table 1) 12 months after the treatment. These patients had not received antimicrobial treatment during this period. Two of these six (Nos 3 and 6) had only fully sensitive strains (Table 1). Patient No. 1 had a tetracycline resistant strain as a majority (all the 20 colonies tested) similar to the T only resistant strain seen in her treatment samples. All the colonies tested from another patient (No. 5) were sulphonamide resistant but otherwise sensitive—he also had had similar flora in the treatment samples in which multiresistant strains were found as a minority fraction. Patient No. 10 had a fully sensitive strain predominating the faecal flora but a T, S, Su resistant strain as a minority (1 of 20 colonies tested). In one of the patients (No. 2) there was a majority flora of tetracycline resistant strains of *Staphylococcus aureus* instead of coliforms. Nine colonies of coliforms tested appeared to be of pattern T, S, Su. The resistance of all the multiresistant strains in the post-treatment samples was transferable: T, S, Su in patient 2, T, S (patient 5) and S, Su (patient 10). Although the number of patients is low, the post-treatment samples seem to indicate that antimicrobial resistance and R factors can persist for a long time after antibiotic treatment.

Examination of the method used

The above findings are based on data from nonselective plates (Method 1). Plating of the samples on tetracycline plates (Method 2) gave very similar results but with much less work. The only difference was a slight increase in the number of tetracycline resistant colonies from 27 to 36% in the pretreatment samples and from 80 to 84% in the treatment specimens as seen in Table 4. On this basis we would recommend the use of selective plates in surveys of the prevalence of antimicrobial resistance in faecal strains.

DISCUSSION

The data of this work demonstrate that long term tetracycline treatment with low doses of 100 mg/day for acne vulgaris alters the resistance pattern of the faecal flora considerably. The mean percentage of persons with tetracycline resistant strains (80%) obtained during treatment differs significantly from that in pretreatment specimens (27%, Table 4). The percentage of persons with multiresistant

TABLE 2. The occurrence of transferable resistance factors in patients in relation to tetracycline treatment

Patient no.	Pretreatment day		Treatment day		Post-treatment year
	7	0	30	60	
1	−	−	−	−	−
2	−	−	+	+	+
3	−	−	−	−	−
4	−	−	−	+	NS
5	−	NS	NS	+	+
6	−	NS	−	+	NS
7	−	−	+	−	−
8	−	−	−	+	NS
9	−	−	+	+	NS
10	NS	NS	−	−	+
11	−	−	−	−	NS
12	−	−	−	−	NS
13	+	+	+	+	NS

TABLE 3. The correlation of transferable resistance factors and multiresistance

Antibiotic sensitivity pattern	No. of strains tested	No. of RTF positive strains
Single resistance (to any drug)	35	7 (20)*
Multiple resistance	25	21 (84)

* Percentage in parentheses.

TABLE 4. The mean percentage of persons excreting strains resistant to tetracycline (T), ampicillin (A), streptomycin (S) and sulphonamide (Su)

	Pretreatment						Treatment					
	T	A	S	Su	mr*	No.†	T	A	S	Su	mr*	No.†
Nonselective media	27	27	18	9	18	440	80	40	40	44	48	500
Selective media	36	27	18	9	18	43	84	32	40	40	44	49

* mr = multiresistant strains; † No. = number of strains tested.

strains was 18% before the treatment and 48% during treatment. The number of patients with transferable R factor increased from one (pretreatment samples) to seven (treatment samples) of thirteen patients (Table 2).

Datta *et al.* (1971) have shown that a therapeutic regimen of 1000 mg/day of tetracycline increases the number of R factors and multiresistant strains. In a report by Hirsch *et al.* (1974) 1000 mg of tetracycline taken orally per day promoted the establishment of a tetracycline resistant bovine *E. coli* strain in the human gut; 50 mg/day instead of 1000 mg/day taken orally was not enough to have this effect. Our data indicate that a very low dose of tetracycline (100 mg/day) is enough to maintain a selective pressure for tetracycline resistant micro-organisms in the intestinal flora.

Datta *et al.* (1971) have shown that *E. coli* becomes resistant after drug treatment due always to the replacement of the original serotype by a different, drug resistant strain. They also reported that tetracycline treatment favours the selection of R factors and drug resistance significantly more often than do ampicillin and sulphonamide. This might be due to the close linkage of genes determining the resistance against tetracycline to the transfer genes in the plasmid (Watanabe, 1963). Another explanation could be the enterohepatic circulation of tetracycline, which probably keeps the drug concentration at a high level in the gut.

Therefore patients treated with tetracycline for acne vulgaris are ecologically an important reservoir and vector for the transmission of R factors, because the treatment lasts for months or even years and they tend to excrete in their faeces many multiresistant strains carrying R factors. Moreover, drug resistance and R factors might survive for a long period after the treatment. These strains are also of potential danger for the patients themselves should they develop for example an ascending urinary tract infection.

ACKNOWLEDGMENTS

This work was supported by a grant from Duodecim (Winthrop) fund. We thank Hannele Kiviaho and Marianne Hovi for technical assistance.

REFERENCES

Anderson, E.S. (1968) The ecology of transferable drug resistance in the *Enterobacteriaceae*. *Annual Review of Microbiology*, **22**, 131.

Datta, N. (1969) Drug resistance and R factors in the bowel bacteria of London patients before and after admission to hospital. *British Medical Journal*, **ii**, 407.

Datta, N., Faiers, M.C., Reeves, D.S., Brumfitt, W., Ørskov, F. & Ørskov, I. (1971) R factors in *Escherichia coli* in faeces after oral chemotherapy in general practice. *Lancet*, **i**, 312.

Ericson, H., Högman, C. & Wichman, K. (1954) A paper disc method for determination of bacterial sensitivity to chemotherapeutic and antibiotic agents. *Scandinavian Journal of Clinical Laboratory Investigation*, **6**, Suppl. 11, 21.

Hartley, C.L. & Richmond, M.H. (1975) Antibiotic resistance and survival of *E. coli* in the alimentary tract. *British Medical Journal*, **iv**, 71.

Hirsch, D.C., Burton, G.C., Blenden, D.C. & Tsutakawa, R. (1974) The effect of tetracycline upon establishment of *Escherichia coli* of bovine origin in the enteric tract of man. *Journal of Applied Bacteriology*, **37**, 327.

Jonsson, M. (1974) On the persistence of R factors in the gram-negative aerobic flora of the human intestine. *Scandinavian Journal of Infectious Diseases*, **6**, 339.

Leading article (1973) Antibiotics in acne vulgaris. *British Medical Journal*, **i**, 65.

Smith, E.L. & Mortimer, P.R. (1967) Tetracycline in acne vulgaris. *British Journal of Dermatology*, **79**, 78.

Watanabe, T. (1963) Infective heredity of bacterial drug resistance. *Bacterial Review*, **27**, 87.

British Journal of Dermatology (1976) **95**, 317.

Pharmacology and Treatment

A double-blind trial of clomocycline in the treatment of persistent palmoplantar pustulosis

JOANNA M.WARD, MARGARET F.CORBETT AND M.J.HANNA*†

Department of Dermatology, Central Middlesex Hospital, and *St John's Hospital for Diseases of the Skin, London

Accepted for publication 5 January 1976

SUMMARY

Sixty patients with persistent palmoplantar pustulosis (PPP) were treated with clomocycline (Megaclor) in a double-blind, cross-over trial in an attempt to establish whether the condition responds to tetracycline. Each patient received 3 months each of clomocycline and placebo in random order. Forty patients completed the trial. Twenty-two failed to respond to either treatment, fifteen improved on clomocycline, two improved on placebo and one improved on both treatments. These results are highly significant ($P = 0{\cdot}003$) and suggest that clomocycline may suppress pustulation in some patients. The twenty-two 'non-responders' were compared with the eighteen 'responders' for sex, age, length of history and associated psoriasis but no significant differences were found. Difficulties in assessment and the possibility of improvement being due to spontaneous remission are discussed. Further follow-up of both groups suggested that clomocycline used over long periods favourably influenced the course of the disease in the 'responder' group.

Persistent palmoplantar pustulosis (PPP) is an intractable condition defying both treatment and investigation. It has been suggested that tetracyclines may help some cases. Thomsen & Østerbye (1973) reported significant improvement in a trial using tetracycline. Wilkinson (1968) stated that tetracyclines were valuable in low dosage and long courses in about a third of cases. A pilot trial was performed by one of us (J.M.W.) using 500 mg tetracycline daily in fifteen patients for from 4 to 14 months: six patients were markedly improved with two apparently cured after 1 year's follow-up; two were dubiously improved; four were definitely unaffected and three failed to attend. Previous experience therefore suggests that at least 50% of patients are likely to remain unaffected by tetracycline. The point at issue is whether tetracycline benefits any cases at all or whether such improvement, when noted, has been fortuitous.

We here report the results of a trial with clomocycline (Megaclor). This is a highly soluble chlortetracycline derivative which is well absorbed and has been shown to be effective in the long-term therapy of acne (Verbov, 1968; Ganpule, 1968). For the purpose of the trial a tablet containing 170 mg of clomocycline was developed in order to overcome difficulties in the formulation of an identical dummy capsule.

---

† Present address: Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000.

PATIENTS

The trial was carried out on patients with an established clinical diagnosis of PPP who were already attending or had been specifically referred to the dermatological out-patients of three hospitals (St John's Hospital for Diseases of the Skin, Charing Cross Hospital and Central Middlesex Hospital). Diagnosis was based on the presence of chronic, recurring, sterile pustulation on the palms and/or soles with characteristic cyclical changes in the pustules from yellow to brown followed by shedding of the dry scale.

Each patient was personally examined by one of the authors at least 2 months previously. They were admitted to the trial if: (1) They were found to have the disease in active form characterized by yellow pustules or brown macules >2 mm in diameter. (2) They had not taken tetracycline within the previous 3 months. They were excluded from the trial if: (1) During the preceding 2 months their disease consisted only of scaling, erythema, and/or tiny vesicles or brown macules <2 mm in diameter. (2) They were pregnant, or likely to conceive, or some other contra-indication to tetracycline was present.

Sixty patients were entered between February 1973 and May 1974 of whom forty-eight were women, mean age 52·4 years ± 12·3 and twelve men, mean age 45·6 years ± 13·8. The patients were told that a treatment was being evaluated which might help some cases and their co-operation for at least 6 months was sought.

METHOD

Two packs were made up for each patient by the manufacturers and labelled with a code number from 1 to 60. One pack contained 3 months supply of 170 mg tablets of clomocycline and the other 3 months supply of dummy tablets of identical appearance containing sugar and starch BPC. The paired packs were randomly labelled A and B using a restricted randomization in tens, so that when treatment was started with pack A neither investigator nor patient knew whether it contained clomocycline or placebo. The patients were asked to take one tablet from pack A three times a day with meals for 2 weeks and then twice a day for 10 weeks. After 3 months they were supplied with pack B, the alternative treatment, to be taken in the same way. Patients were asked to return any tablets left over at the end of each treatment period. A known excess of tablets had been included in order to check on tablet consumption.

Local treatment

At the start of the trial emulsifying ointment or dilute Betnovate 1:4 in petrolatum were used where-ever possible. Six patients were left on full strength fluorinated steroids topically. Plastic occlusion, however, was always discontinued.

Assessment

Patients were seen by an observer at intervals of 6 weeks. In addition, the first thirty patients seen at St John's Hospital were independently assessed by a second observer for the first 3 months of the trial. At the first visit the extent of the disease and the approximate location and number of pustules and brown macules were charted on an outline diagram of the palms and soles. The state of the surrounding skin was also recorded. At each subsequent visit a similar record was made and the patients were carefully questioned about pustulation in the intervening period and also about symptoms of itching and soreness. By comparison of diagrams the pustulation was graded as: more, unchanged, less, much less, or none, at each visit. Erythema, scaliness and the area involved were considered together and graded as: worse, no change, less or clear. From these data an overall conclusion was determined as: worse, no change or better, both at 6 and 12 weeks. At 12 weeks those patients who were graded as improved were classified as 'responders' whilst those in whom no change or worse had been recorded were classified as 'non-responders'.

The significance of the effect of treatment was judged by the numbers of patients who responded to one treatment but not to the other. In the absence of effective treatment half the patients are expected to prefer each of the treatments and the departure from this proportion can be tested using the binomial distribution (Armitage, 1975).

RESULTS

Assessment of PPP by the method described was found to be practical and reproducible. In the thirty patients who were separately judged by two observers there was complete agreement in fifty-eight of sixty assessments made.

Of sixty patients entering the trial twenty failed to complete the treatment. Of the remaining forty patients: twenty-two failed to respond to either treatment, i.e. showed no preference (= 'non-responders'); fifteen improved on clomocycline and failed to respond to placebo, i.e. showed preference for clomocycline; two improved on placebo and failed to respond to clomocycline, i.e. showed preference for placebo; one improved on both treatments, i.e. showed no preference.

Thus seventeen patients expressed a preference for one of the treatments and of these fifteen showed a preference for clomocycline and two for placebo. All the fifteen patients who responded to clomocycline either failed to respond to placebo if this was given first or relapsed on placebo when this was given second.

The probability of getting by chance fifteen preferences for clomocycline from a total of seventeen is very small ($P = 0{\cdot}003$), i.e. there is a highly significant difference between the effectiveness of clomocycline and placebo in a proportion of patients who suffer from PPP. Comparison of eighteen 'responders' with twenty-two 'non-responders' failed to show any obvious clinical reason for the different effects of treatment in the two groups (Table 1).

Analysis of twenty patients who failed to complete

Six patients did not attend, four after the first visit. The remaining fourteen discontinued with side

TABLE 1. Comparison of 'non-responders' and 'responders'

	Non-responders	Responders
Total no.	22	18
Age ± s.d. (years)	52·9 ± 14·9	49·5 ± 11·2
Length of preceding history (years)	3·5 ± 3·6	3·3 ± 4·3
Sex M:F	3:19	6:12
Associated psoriasis	6	3
Family history of psoriasis	4	2
Past history of spontaneous remission	3	2
Distribution of lesions:		
Palms only	0	1
Soles only	8	6
Palms & soles	14	11

effects: one of these was on placebo and complained of heartburn; while the thirteen on clomocycline complained of nausea and vomiting (6), vaginal thrush (1), constipation (1), heartburn (1), miscellaneous symptoms (4).

Results of further treatment and follow-up in forty patients completing the trial

The condition of the group of patients who appeared to respond to clomocycline was compared to that of the group of 'non-responders' after a period of follow-up lasting from 6 months to 2½ years—thirty-three patients being followed for 1 year or more and seven for 6 months. Comparison of these two groups shows that a significant proportion of those who initially responded had maintained their improvement during the period of follow-up, i.e. nine of eighteen as compared to three of twenty-two 'non-responders'. Nine of the 'responders' had from 2 to 6 months further treatment with tetracyclines. Twenty-eight patients were still pustulating 1 to 2 years later, nineteen from the non-responding group and nine from the responding. Six of the latter had relapsed without treatment within 3 months of the end of the trial and three remained better as long as they were given further treatment with clomocycline capsules but relapsed when it was stopped. Ten of the nineteen 'non-responders' had had further treatment with tetracyclines to no avail.

These results tend to confirm a difference in response to tetracyclines between the two groups and suggest that pustulation will remain suppressed in 'responders' whilst treatment is continued and possibly afterwards in some cases.

DISCUSSION

Assessment of treatment is difficult in PPP because of its fluctuating course. The importance of pustulation as the main index of activity is corroborated by the natural history of the disease. When spontaneous remission occurs gradually fewer pustules are produced, itching and soreness diminish and there often follows a long period in which the skin remains clinically abnormal, e.g. red and/or scaly but without pustulation. The actual clinical picture in this condition varies considerably from patient to patient, but in any one individual it is often strikingly constant regarding the area involved, morphology of pustules and amount of erythema and hyperkeratosis. This, together with the need to treat as many patients as possible, suggested the use of patients as their own controls in a crossover design. The length of the treatment period chosen depended on three factors. Firstly in many patients, and especially those with palmar lesions, the pustules appear in crops and there may be complete clearing in between. It was important, therefore, to make the treatment periods sufficiently long to encompass fresh crops and to include the patients' account of the period between visits in the assessment. Secondly, data from the pilot series and from the patients treated by Samman (personal communication) suggested that a minimum of 6 weeks treatment was necessary to produce any change, and that relapse was correspondingly slow. Hattel & Søndergaard (1974) considered the failure of hydroxyurea given for 3 weeks to patients with PPP might be due to a too short treatment period. The 1 month treatment periods of Thomsen & Østerbye (1973) may also have been too short to give a true picture. Thirdly, it was considered that the carry over effect from clomocycline, when it preceded placebo in the cross-over design, would be allowed for by the use of a 3 month treatment period.

Twenty-two of forty patients completing this trial were unaffected during the 6 months treatment suggesting that at least 50% of patients are likely to be unresponsive to tetracyclines. Eighteen patients improved during treatment: fifteen improved on clomocycline and three on placebo including one who improved on both. Statistical analysis is consistent with the view that pustulation was suppressed by clomocycline in these fifteen patients although the number of patients involved is small. Alternatively one may consider whether these fifteen patients are likely to have improved spontaneously. The incidence of spontaneous remission would appear to be low in this condition. Hellgren & Mobacken (1971) stated that 75% of patients were still pustulating after 5 years. Enfors & Molin (1971) found 75% of 248 patients to have symptoms more than 10 years after onset. Of eighty-two patients they had under observation for 4–6 months 7% had cleared. During the 6 months of our trial the three placebo reactors may represent spontaneous remissions as may also the one non-responder who subsequently cleared giving a comparative figure of 10%. If, however, all those who responded over the 6 months of the trial are considered to have improved spontaneously an incidence of 45% healing obtains which seems highly unlikely. In addition the forty patients who completed this trial were specifically asked for a past history of spontaneous remission defined as clearing of one or more limbs for 3 or more months—only five patients gave such a history (12·5%). Further follow-up of our patients adds to the evidence for clomocycline exerting a beneficial effect in some cases.

It is well known that patients may vary widely in their response to drugs. This may be due to the disease being treated, the responsiveness of the tissues to the drug or the plasma concentration of the drug (Rawlins, 1974).

No significant difference could be found in the clinical features of responders and non-responders. However, PPP may not be a homogeneous disease entity. Some patients show a more eczematous response with severe itching, vesicle formation and often lichenification in addition to pustulation. Nine patients showed these features. A further group of patients with PPP may conform to the description by Andrews & Machacek (1935) of pustular bacterids. This is probably a rare self-limiting variant and only one of our patients appeared to fall into this category. These groups may have a different pathogenesis from the main bulk of PPP.

Nothing is known about tissue responsiveness to tetracyclines in this condition. A variation in serum level between the two groups seems unlikely, but this possibility is being further investigated.

A disappointingly large number of patients failed to complete the trial. In particular, it was unexpected to find so many (twelve) complaining of nausea, vomiting and heartburn; seven of the eight of these who had to withdraw from the trial were taking clomocycline. In previous clinical trials using this drug, Ganpule (1968) comparing its effect with that of oxytetracycline found two of twenty-five patients to have severe nausea and vomiting with clomocycline as compared to three patients with severe diarrhoea and five with mild diarrhoea on oxytetracycline. A further trial is at present being carried out with oxytetracycline.

In conclusion, although the numbers involved are too small to be entirely conclusive, this trial has produced good evidence that some cases of PPP benefit from oral tetracyclines.

ACKNOWLEDGMENTS

We are grateful to Dr O.L.Scott, Dr H.T.H.Wilson and the physicians of St John's Hospital for kindly allowing us to treat their patients. We should like to thank Dr J.J.Misiewicz for help in planning the trial and, together with Dr E.Cronin, criticizing the manuscript. Our thanks are also due to Mr M.J.R.Healy for help with the statistics, Dr A.G.Thomson of Pharmax Limited for technical advice and supplying clomocycline and placebo and Miss Sheila Lambert for secretarial assistance.

REFERENCES

ANDREWS, G.C. & MACHACEK, G.F. (1935) Pustular bacterids of the hands and feet. *Archives of Dermatology & Syphilology*, **32**, 837.

ARMITAGE, P. (1975) *Sequential Medical Trials*, 2nd edn. p. 41. Blackwell Scientific Publications, Oxford.

ENFORS, W. & MOLIN, L. (1971) Pustulosis palmaris et plantaris. *Acta dermato-venereologica*, **51**, 289.

GANPULE, M. (1968) Clomocycline and oxytetracycline in acne vulgaris. *Practitioner*, **201**, 479.

British Journal of Dermatology (1976) 95, 323.

Co-operative double-blind trial of an antibiotic/corticoid combination in impetiginized atopic dermatitis

GERALD N.WACHS* AND HOWARD I.MAIBACH

University of California School of Medicine, San Francisco, California 94143, U.S.A.

Accepted for publication 14 January 1976

SUMMARY

This double-blind study of an antibiotic corticosteroid combination, the antibiotic alone and the corticosteroid alone, compared their clinical and microbiological effect in infected atopic dermatitis. Combination therapy reduced the mean scores for infection, inflammation and overall severity to a greater extent than the antibiotic or corticosteroid alone. The clinical and microbiological data are discussed in terms of relevance to clinical use.

Dermatological opinion is divided on the efficacy of antibiotic/corticosteroid combinations compared to a corticosteroid alone in the treatment of steroid-responsive dermatoses with signs of infection. Blank (1968), Davis, Fulglum & Taplin (1968), Larsen (1968), Lloyd (1974) and others suggest that corticosteroids alone should be used in the treatment of impetiginized dermatoses. Others feel that the advantages of adding an antibiotic to a topical corticosteroid outweigh the risks of sensitization (Esterly & Markowitz, 1970; Sulzberger, 1972; Kligman, 1973; Marples, Rebora & Kligman, 1973; Wachs, Clark & Hallett, 1973; Carney, 1963; Carpenter, 1972).

A significant aspect of this problem is the observation by Leyden, Marples & Kligman (1974) of high concentrations of *Staphylococcus aureus* occurring on lesions of atopic dermatitis even in the absence of clinical signs of infection. Blank (1973) also suggested this.

The present communication reports a twelve investigator† co-operative controlled double-blind trial of the therapeutic effects of a gentamicin betamethasone valerate cream preparation compared with each of its ingredients alone in the treatment of infected dermatitis.

MATERIALS AND METHODS

A randomized, double-blind technique was employed, precautions being observed to preserve the

---

* Address: Dr G.N.Wachs, 2000 Galloping Hill Road, Kenilworth, New Jersey 07033, U.S.A.

† Stuart H.Bender, M.D., Samuel M.Bluefarb, M.D., Harvey J.Cohen, M.D., Robert F.Dickey, M.D., Phillip Frost, M.D., John A.Kenney, Jr., M.D., S.William Levy, M.D., Jack E.McCleary, M.D., Franklin Pass, M.D., William E.Rosenberg, M.D., J.Graham Smith, Jr., M.D., Malcolm Spencer, M.D.

'blinding' of both patients and therapists. To aid in achieving uniformity in diagnosis and in grading of responses to treatment, the investigators assembled for study and discussion of colour slides illustrating the pre- and post-treatment appearance of lesions of infected atopic dermatitis. Each investigator separately rated the severity of each patient. With this training the ratings became reasonably uniform. Data were recorded on a standardized report form (Table 1).

TABLE 1. Assessment schedule used

Evaluation	Method	
	Score	Interpretation
Overall severity	0	Complete absence
Degree of inflammation	1	
Degree of infection	2	Slight
Individual signs and symptoms: erythema, pruritus, pustules, crusting, exudation, vesiculation and lichenification	3	
	4	Mild to moderate
	5	
	6	Moderate to severe
	7	
	8	Severe
	9	
	10	Very severe—worst case ever seen
Results of treatment Overall evaluation	Excellent	Complete clinical control of condition (75% or better)
	Good	Moderate clinical control of condition (50–74%)
	Fair	Partial control of condition (25–49%)
	Poor	Less than 25% improvement or worsening of the condition
Bacteriology	Culture	

Each physician planned to empanel fifteen patients, with no restrictions as to the race and sex of patients. The enrollment criteria were so rigid that only eighty-three patients were actually enrolled. Pregnant patients, children under 1 year of age, patients with known renal disease and patients with initial symptoms severe enough to warrant the use of systemic antibiotics were excluded. For patients receiving a systemic antibiotic or corticosteroid at the time of selection for the study, a 3-week drug-free 'wash-out' period was required; for those receiving prior topical therapy, 1 week without treatment.

Patients under the care of an individual investigator were randomly assigned so that five were to receive the plain antibiotic, five the plain corticosteroid and five the combination cream. Medication was applied three times daily; no occlusive dressings were used. Patients were asked not to use medicated soaps throughout the study period. No other systemic or topical antibiotics, corticosteroids, or antihistamines were permitted in the care of these patients; those for whom such treatment became necessary were considered 'treatment failures'.

Specimens for culture and sensitivity were obtained prior to treatment, on the 8th day and again on the 22nd day. The cultures were performed on Tryptone Soy Agar blood agar and thioglycollate broth. For measurement of sensitivity the disc method of Kirby–Bauer was used. A urinalysis was performed at every visit.

In an attempt to quantify a subjective evaluation each investigator graded the following at each visit: (1) An overall or 'global' assessment of the condition, overall severity; (2) Degree of inflammation; (3) Degree of infection. Additionally, the following list of signs and symptoms were graded: erythema, pruritus, pustules, crusting, exudation, vesiculation and lichenification. Investigators graded each patient with respect to each symptom at each visit, according to the rating scale shown.

In order to be accepted for the study, a clinical diagnosis of infected atopic dermatitis had to be established with an initial overall rating of 5 or higher ('moderate to severe'). Follow-up visits were scheduled for the 4th, 8th, 15th and 22nd days of treatment. At each follow-up visit the investigators also made an overall evaluation of each patient's response to treatment in addition to the evaluations previously described. The standard commercially available betamethasone valerate (Betnovate) cream base was employed for all formulations.

Eighty-three patients were enrolled. There were four dropouts. Twenty-five were treated with the combination preparation, twenty-seven received only betamethasone valerate and twenty-seven received only gentamicin.

Combination therapy reduced the mean scores for infection, inflammation and overall severity of the seventy-nine patients from the initial range of 5·1–6·1 down to the range of 0·00–1·0, while each of the two agents, given alone, had a less marked effect (Table 2). Analyses of variance of these data yield probabilities which were significant for betamethasone valerate combination vs gentamicin in all cases but only for infection in betamethasone valerate combination vs betamethasone valerate alone (Table 3).

All patients enrolled were clinically judged to have moderate to severe impetiginized atopic derma-

TABLE 2. Scores for each treatment group

Treatment	BV/G (Corticosteroid/ antibiotic)	BV (Corticosteroid)	G Antibiotic
Degree of infection			
Initial	5·1	5·2	5·5
Final	0·0	1·4	1·8
Degree of inflammation			
Initial	5·8	5·9	6·2
Final	0·7	1·4	3·8
Overall evaluation of severity of condition			
Initial	6·1	6·1	6·6
Final	1·0	1·8	4·2

TABLE 3. Probabilities associated with treatment differences

	Rx-BV/G vs Rx-BV (*P*)	Rx-BV/G vs Rx-G (*P*)
Degree of infection	0·05	0·01
Degree of inflammation	0·20	0·01
Overall severity	0·20	0·01

No initial differences were found.

titis. Nevertheless, not all the initial cultures demonstrated the presence of bacteria and not all the species identified are customarily regarded as pathogens in otherwise uncomplicated dermatoses. A list of organisms found on initial culture is presented in Table 4. Evaluation of the culture results shows how accurate the clinicians were in their original diagnosis of infection.

TABLE 4. Bacteria isolated at initial culture

Organism	Patients admitted to treatment group			
	Combination BV/G	Betamethasone BV	Gentamicin G	Total
Staphylococcus spp.	21	20	22	63
Beta-haemolytic strep	—	2	—	2
Pseudomonas spp.	1	—	—	1
Proteus spp.	—	—	1	1
Non-pathogens	3	5	3	11
No growth	—	—	1	1
Totals	25	27	27	79

TABLE 5. Final culture results compared with initial results

Initial	Final							
	Staph. (coag. +)	Staph. (coag. −)	Proteus	Pseud.	Not done	No growth	Non-path	Total
Combination treatment								
Pseudomonas species	—	—	—	—	—	1	—	1
Staph. aureus (coag +)	4	—	—	—	1	5	3	13
Staph. species (coag −)	—	2	—	—	1	4	1	8
Non-pathogens	—	—			—	1	2	3
Sub-total	4	2	—	—	2	11	6	25
Steroid alone								
Staph. aureus (coag +)	3	1	1	—	1	3	3	12
Staph. species (coag −)	—	3	—	—	1	2	2	8
Beta-haemolytic strep.	1	—	—	—	1	—	—	2
Non-pathogen	—	—	—	1	—	3	1	5
Sub-total	4	4	1	1	3	8	6	27
Gentamicin alone								
Proteus	—	—	—	—	—	1	—	1
Staph. aureus (coag +)	4	—	—	1	5	2	3	15
Staph. species (coag −)	—	—	—	—	—	1	6	7
No growth	—	—	—	—	—	1	—	1
Non-pathogen	—	—	—	—	1	1	1	3
Sub-total	4	—	—	1	6	6	10	27
Totals	12	6	1	2	11	25	22	79

From a purely bacteriological point of view, and without regard to the clinically-judged outcomes of treatment, it is of interest to pursue changes in the results of cultures with treatment. For each treatment, the results of initial cultures are tabulated against the results of final cultures (Table 5).

Of twenty-five patients treated with the combination cream, twenty-two initially yielded a pathogen. After 3 weeks, twenty-three patients had a final culture; six remained positive.

Of the twenty-seven patients treated with plain corticosteroid, twenty-two cultures initially demonstrated the presence of a skin pathogen. After 3 weeks of treatment, twenty-four patients were recultured; ten pathogens were identified. (One of the final culture pathogens was recovered from a patient whose initial culture contained only non-pathogenic organisms.)

Of the twenty-seven treated with gentamicin alone, twenty-three initially cultured a pathogen. After 3 weeks, twenty-one patients had a final culture. Five were still positive for pathogens.

Paired comparison of these data using the Fisher Exact Probability Test demonstrate that the number of remaining pathogens in each of the three treatment groups is not significant ($P > 0{\cdot}30$). The plain corticosteroid group had the largest number of final culture pathogens. This group also showed the largest number of cases of persistence of *Staph. aureus*. The gentamicin treatment showed the fewest (4 of 17) (with an additional case showing pseudomonas). Streptococci were cultured in two patients; the organisms were not resistant to gentamicin.

The bottom line of Table 5 summarizes the bacteriological outcome of the study with respect to organisms generally assumed to be skin pathogens. At the end of treatment, twenty-one of sixty-eight cultures remained pathogenic. Thus, Table 5 indicates the relative efficacy—from a bacteriological point of view—of the three modalities.

TABLE 6. Summary of physicians' final evaluation of treatment

	Treatment group		
Result	Combination	Steroid	Gentamicin
Excellent	18 (72)*	15 (56)	8 (30)
Good	5 (20)	5 (18)	1 (4)
Fair	2 (8)	4 (15)	7 (26)
Poor	0 (0)	3 (11)	11 (41)

* Percentages in parentheses.

Table 6 presents the totals of the physicians' final evaluations of the results of treatment. The data favour combination antibiotic/steroid therapy in the treatment of impetiginized atopic dermatitis over treatment with plain antibiotic ($P < 0{\cdot}0001$) (Wilcoxin 2 Sample Test). There was no statistically significant difference between antibiotic/steroid and plain steroid ($P > 0{\cdot}13$).

DISCUSSION

In the judgment of many clinicians, topical antibiotics may be sensitizing and do not necessarily enhance the effectiveness of corticosteroids. Others believe, equally firmly, that treatment with corticosteroids alone is potentially dangerous in that they may alter the body's natural resistance, mask the clinical manifestations of infection and allow overgrowth of pathogens.

Recent *in vivo* and *in vitro* studies by Raab (1971; 1973) showed some antibiotic-corticosteroid combinations to be more effective than their individual components in topical therapy of infected dermatoses. The antibacterial activity of gentamicin when combined with betamethasone valerate remained unimpaired and was superior to the other preparations studied. A study of these same agents by Kligman (1971), employing quantitative bacterial counts, indicated a high degree of effectiveness. There have been other studies which favour this combination, but none has been well controlled (Nakai, 1969; Bruni, 1968; Sosa, 1966; Tonkin, Politer & Robinson, 1966).

Both clinically and bacteriologically, the results appear to favour the use of the combination cream over gentamicin alone. The data are not statistically significant at every point. It is possible that a larger study would demonstrate further statistical significance. This pharmacological assay should be considered in context as an attempt to clarify the drugs' degree of effect, rather than suggesting that one form is manifestly better than the other. That degrees of difference are demonstrated (clinically and microbiologically) does not alone signify their general usefulness; toxicological and other considerations must be weighed against efficacy. What is the relevance of these data to clinical medicine as practised by the dermatologist or the general physician? The final judgment remains subjudice; hopefully this controlled study provides one piece for this judgment.

REFERENCES

Blank, H. (1968) Personal communication.

Blank, H. (1973) Personal communication.

Bruni, L. (1968) Association of betamethasone valerate and gentamicin sulfate in the treatment of inflammatory dermatoses. *Minerva Dermatologica*, **43**, 89.

Carney, R.G. (1963) Topical use of antibiotics. *Journal of the American Medical Association*, **186**, 646.

Carpenter, C.L. (1972) Combined steroid–anti-infective topical therapy in common dermatoses (scientific exhibit). *American Academy of Family Physicians*.

Davis, C.M., Fulglum, D.D. & Taplin, D. (1968) The value of neomycin in a neomycin–steroid cream. *Journal of the American Medical Association*, **203**, 136.

Esterly, N.B. & Markowitz, M. (1970) The treatment of pyoderma in children. *Journal of the American Medical Association*, **212**, 1667.

Herrell, W.E. (1968) Editorial. *Clinical Medicine*, **19**, 19.

Kligman, A. (1971) Personal communication.

Kligman, A. (1973) In praise of what's old. *Dermatology News*, **7**, 7.

Larsen, W.G. (1968) Topical neomycin. *Archives of Dermatology*, **97**, 202.

Leyden, J.J., Marples, R.R. & Kligman, A.M. (1974) *Staphylococcus aureus* in the lesions of atopic dermatitis. *British Journal of Dermatology*, **90**, 525.

Lloyd, K.M. (1974) Letter to the editor. *Archives of Dermatology*, **109**, 410.

Marples, R.R., Rebora, A. & Kligman, A.M. (1973) Topical steroid–antibiotic combinations. *Archives of Dermatology*, **108**, 237.

Nakai, Y. (1969) Increase in bacterial population of skin surface by ODT and the effect of addition of antibiotics to topical preparations. *Acta Dermatologica*, **64**, 25.

Raab, W. (1971) The interaction of corticosteroids and antimicrobial agents used in topical therapy. *British Journal of Dermatology*, **84**, 582.

Raab, W. & Windisch (1973) Corticosteroids vs antibiotics. *Dermatologica*, **185**, 400.

Sosa, C.B. (1966) Betamethasone valerate and gentamicin sulfate combination in the treatment of some dermatoses. *Medical Review of Mexico*, **46**, 501.

Sulzberger, M.B. (1972) Combined drugs important in skin disease treatment. *Skin & Allergy News*, **2**, 2.

Tonkin, M., Politer, W.M. & Robinson, R.G. (1966) Percutaneous absorption of a corticosteroid in combination with an antibiotic. *South African Medical Journal*, **40**, 1071.

Wachs, G., Clark, R. & Hallet, J. (1973) Are topical steroid-related superinfections a myth? *Cutis*, **10**, 22.

British Journal of Dermatology (1976) **95**, 329.

Brief Communications

Corticotrophin and vitiligo (preliminary observations)

B. B. GOKHALE AND TARA B. GOKHALE 322 *Mahatma Gandhi Road,*
Poona 411 001, *India*

Twenty-seven patients with vitiligo have been treated with parenteral long-acting corticotrophin. Sixteen were male and 11 female. The duration of the disease was from 2 to 16 years. Sixteen patients had been treated unsuccessfully at the clinic with psoralens. Long-acting ACTH was administered intramuscularly twice-weekly in doses of 25–40 I.U. depending on the age of the patient. Ten to twelve injections were given followed by a break of 2–4 weeks. Such courses were repeated as required, the maximum given being four courses.

The patients who had previously been treated noted a quicker and better response than with psoralens. The results were evaluated at the end of 6 months. 80% repigmentation occurred in 16 cases, 50% in 6 cases, 20% or less in 4, and no improvement in one case. The duration of this repigmentation remains to be assessed. Although there are potential hazards to such treatment, no side-effects were observed in this series.

Muscle herniation of the lower legs

JULIAN VERBOV *Sefton General Hospital, Liverpool L15 2HE*

Asymptomatic small muscle herniae of the lower legs are said to be not uncommon in those whose legs are exposed to severe chronic strain, such as athletes, heavy manual workers, mountain climbers and soldiers. However, for a patient to present at a clinic with the condition is most uncommon. Herniation of muscle (tibialis anterior) takes place through hiatuses in the deep fascia where it is

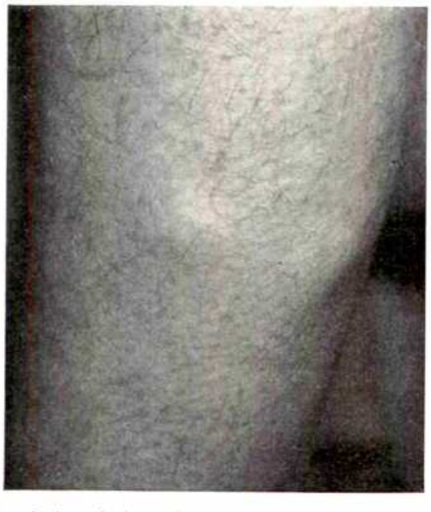


FIGURE 1. Muscle herniation of the left lower leg—two herniae visible.

perforated by the smaller cutaneous vessels or through fascial splits. The usual differential diagnosis is varicosities.

A young woman aged 20 years presented with the condition. She worked as a canteen worker (standing all day). She gave a 14-month history of lower leg discomfort particularly towards the end of the day. Discomfort was associated with the appearance of two lumps over the antero-lateral aspect of both lower legs. On examination, although nothing abnormal was visible on standing, when she sat with her knees crossed two almost symmetrical soft swellings were palpable over each lower leg antero-laterally (Fig. 1). The lower herniation was situated about 15 cm above the lateral malleolus on the left and 13 cm above the lateral malleolus on the right. The upper herniation was both higher and more medially situated than the lower one on each leg and the distance between them was about 4 cm greater on the right than on the left leg. Elastic support stockings were recommended.

This condition, in which single or multiple unilateral or bilateral herniae may occur, has been well-described (Ihde, 1929; Kitchin & Richmond, 1943; Obermayer & Wilson, 1951) but may be unfamiliar to many dermatologists.

REFERENCES

IHDE, H. (1929) On muscular hernia of the leg. *Acta chirurgica Scandinavica*, **65**, 97.

KITCHIN, I.D. & RICHMOND, D.A. (1943) Multiple muscle herniae. *British Medical Journal*, **i**, 602.

OBERMAYER, M.E. & WILSON, J.W. (1951) Fascial hernias of the legs. *Journal of the American Medical Association*, **145**, 548.

Canadian-Irish dermatological meeting

For their 51st Annual Congress, the Canadian Dermatological Association travelled to Dublin and held a joint meeting with the Irish Dermatological Society from 30 April to 4 May. More than one hundred Canadians took part with virtually every dermatologist from both Eire and Northern Ireland under the joint presidencies of Art Hudson (Canada) and Desmond Burrows (Belfast).

The meeting started with an outstanding case presentation of patients at St Vincent's Hospital. They included the first cases of lipoid proteinosis seen in Ireland; severe pityriasis lichenoides acuta in a child; three siblings with cutis laxa; familial actinic prurigo; ichthyosis congenita with a possible epithelioma; sclerodermoid porphyria cutanea tarda aggravated by fluorescent lighting; necrobiosis lipoidica of the scalp; Zurhelle's naevus lipomatosus; a most unusual and extensive vascular abnormality and cutaneous atrophy in a child; and a remarkable progressive histiocytosis with lung involvement.

One morning session was devoted to papers on contact dermatitis. Mitchell (British Columbia) gave an account of the steadily increasing problem of parthenium weed dermatitis which is producing such dermatological devastation in large areas of India. It has now covered more than half a million acres in central India and shows no sign of being halted. No herbicides have been found to be sufficiently selective. Calnan (London) illustrated some of the diverse manifestations of allergic sensitivity to colophony rosin, both industrial and domestic, including ointments.

Rogers and Burrows (Belfast) described a group of workers with dermatitis in a milk testing laboratory where potassium dichromate is used with mercuric chloride to stabilize milk samples prior to fat analysis. Nethercott (Toronto) drew attention to the important new dermatitic hazard from light-sensitive acrylates used in inks for metal printing and printed circuits in industry. Corbett (Belfast) reported an outbreak of irritant dermatitis from spin finishes used in the manufacture of nylon fibres; and Dr Burrows made a plea for greater care in evaluating the degree of disability in patients with occupational dermatitis.

Farber (Palo Alto) gave major presentations on the photochemotherapy trials for psoriasis and a review of cytostatic agents used over the past ten years. Serri (Pavia, Italy) reported his studies on insulin metabolism in psoriasis. Rook (Cambridge) discussed his own experience with alopecia areata, particularly in relation to Ikeda's work on associated abnormalities, atopy and inheritance. He also indicated how one should study the multifactorial aetiology of diffuse alopecia in women.

Dean (Dublin) gave an expert review of the porphyrins, and there were several papers on bullous eruptions. Other subjects covered included IgE in atopic eczema, the role of atopy in contact dermatitis, and the use of benzoyl peroxide in decubitus ulcers.

As one might expect the social programme was scintillating—a reception and dinner at the National Gallery, a Eugene O'Neill play at the Abbey, a dinner and cabaret at Jury's and the President's Banquet at the Shelbourne Hotel. No account of this historic meeting would be complete without a word of praise for Mrs Fitzgerald and her staff of the Irish Medical Association who were responsible for the immaculately smooth running arrangements.

C.D. CALNAN

British Journal of Dermatology (1976) **95**, 332.

Book Reviews

Handbuch der Haut- und Geschlechtskrankheiten. J.JADASSOHN (1976) Ergänzungswerk. III/3A *Nicht Entzündliche Dermatosen III. Gut- und Bosartige Neubildungen der Haut. Sclerosen.* Berlin: Springer-Verlag. Pp. 746, illustrated. Price US $196.80.

This is the first of the two volumes that, under the editorship of the late H.A.Gottron and his successor, G.W.Korting, are to bring up to date the tumour section of the Jadassohn *Handbuch* first published 40 years ago.

The contents range from the simple tumours of the mesodermal components of the skin through the benign epidermal and adnexal tumours to haemangiopericytoma and basal cell epitheliomas in all their variety. Somewhat surprisingly, the final chapter deals with scleroderma and scleroderma-like conditions.

Medical knowledge has advanced immeasurably since first publication of the Jadassohn *Handbuch*, and dermatology has kept pace with other branches of medicine. Perhaps nowhere is this more striking than in our approach to the conditions that are the subject of this volume. Histogenesis and pathophysiology, supported by histochemical findings and electron microscopy, have made it possible better to understand the basic biology and clinical behaviour of the known tumours of the skin, and this in turn has led to a more rational approach to therapy as well as to discovery and description of new entities.

The fundamental changes in existing views on the histogenesis of basal cell epitheliomas and the far reaching consequences of these changes are a case in point. As a result of work on epithelial regeneration and 'stripping' (Pinkus; Gimbel *et al.*) the old, rigid division between 'basal cell' and 'squamous cell' origin of tumours became untenable; however, the undoubted clinical and histological differences between these tumours had to be explained.

It is now agreed that basal cell epithelioma is always composed of an epithelial (ectodermal) and stromal (mesodermal) element. The presence of stroma is necessary for nidation—hence the inability of basal cell epithelioma to metastasize. Thus the tumour, while locally invasive and often highly destructive, nevertheless hovers in the 'grey zone' of Pinkus between cancers and non-cancers.

K. Holubar marshals the facts and laboratory and clinical data that led to this viewpoint with admirable clarity; with equal ease he describes and discusses all the varieties of basal cell epithelioma, their classification, appearance, histochemistry, ultrastructure, etc. Current modalities of treatment are described and assessed. Calcifying epithelioma of Malherbe and basal cell naevus complement the chapter on basal cell epithelioma.

It would be impossible to quote or enumerate the wealth of information this book contains—one can but admire the skill with which it is imparted. The meticulous attention to detail does not detract from the ability of all the contributors to give a bird's eye view on the subjects with which they deal, whether it is what they call the 'saga' of haemangiopericytoma, the premalignant fibro-epithelioma of Pinkus and its relationship to the fully developed organoid basal cell epithelioma or the problem of intra-epithelial basal cell tumour of Borst–Jadassohn (it *cannot* exist as there is no stromal component).

The chapter on the scleroses is impressive in its comprehensiveness and clarity of presentation. All the clinical and pathophysiological aspects are fully described and current methods of therapy discussed.

Each chapter in this book starts by clearly defining its scope and terms and is followed by exhaustive, well arranged bibliography. The reviewers particularly liked the ease with which literature relating to each sub-division could be found by consulting the table of contents. It is a pity, however, that in their endeavour to quote extensively from the existing bibliography, the authors at times make it difficult, if not impossible, to reach a definite conclusion as to the appearance or classification of a particular condition, nor do they make it easier by often refraining from expressing their views.

The index is inadequate for a book of this scope, as it lacks detailed subject reference. The work is rather sparsely illustrated and in general the quality of reproduction of the clinical photographs and micrographs does not match up to the standard one has come to expect from a Springer publication.

In spite of these relatively minor drawbacks, this is an excellent book which will amply repay the effort and time taken to read it.

It is not a book for a beginner, but will be enjoyed by dermatologists eager to understand the conditions they deal with. It should be on the shelves of every serious dermatological library.

J.A.MILNE
I.LOMINSKA

Histological Typing of Skin Tumours. No. 12 in the series of the International Histological Classification of Tumours. Edited by SELDAM and HELWIG (1974) Geneva: World Health Organization.

This short publication edited by Seldam and Helwig in collaboration with Sobin and Torloni, attempts a simple classification of skin tumours. There are translations of the classification into French, Russian and Spanish.

The classification is not a detailed one, and perhaps could not be recommended for a specialist department in skin histopathology. However, for routine pathology departments throughout the world the classification given here is a starting point, particularly in regard to uniformity when collecting statistics from different countries.

Perhaps the most useful part of the book is the section of definitions and explanatory terms, which gives a thumb-nail histological description, with accompanying figures, for the main categories of skin tumours.

The book contains 176 histological figures in glorious technicolour. Most of these illustrate the points they attempt to make, but it could not be said that the colour reproduction is very good, and there is too much emphasis on sickly shades of pink and violet.

E.WILSON JONES

Current Dermatologic Management. Edited by STUART MADDIN (1976) London: Henry Kimpton Ltd. Pp. 404, illustrated. Price £21.75.

This book is divided into three parts, the first on practical procedures, the second on the therapeutic management of dermatological conditions and the third part is a comprehensive index of drugs. The contributors, and there are over 200 of them, are experts of international repute and though mainly from North America, there are many from the U.K. and from other parts of the world. In this second edition, the authors have extensively revised the whole book and there are many additions. Of particular use is the inclusion of a precise delineation of each cutaneous disorder before the description of its therapy. These brief descriptions are as insets in the text and are most helpful. Treatments are always changing and will always be subject to controversy, but the therapies that are advocated in this book are orthodox and in general current use and for me, there were no surprises. A minor point was that in the treatment of discoid lupus erythematosus no mention was made of the use of antimalarials, except briefly in Part 3 under Quinacrine. Perhaps these useful drugs, like arsenic, have been discarded into the dermatologists' therapeutic waste-bin?

The drug index is very comprehensive and Dr Brown has done an excellent job in compiling it. The chemistry, mode of action, absorption, distribution and excretion of the drugs are discussed as well as their side-effects and their most important interactions. The dermatological indications for the use of the drugs are also mentioned.

Of particular value is the first part of the book that covers dermatological procedures. Few dermatological text-books adequately describe these practical procedures and there are excellent accounts on pinch grafts for leg ulcers and on autografts in the treatment of alopecia.

This is to be recommended for the practising dermatologist and is a book which should be on the desk in the consulting room rather than on a library shelf.

S.S.BLEEHEN

Cancer in Children. Clinical Management. Edited by H.J.G.BLOOM, J.LEMERLE, M.K.NEIDHARDT and P.A.VOUTE (1975) Berlin: Springer-Verlag. Pp. 317, 121 figures, 70 tables. Price U.S. $14.20 (soft cover).

As its title suggests, this book provides an excellent up-to-date review of the clinical management of cancer in children. The chapters are well written by experts in the field, so that the book as a whole provides a good example of European co-operation in medicine.

The cutaneous manifestations of the malignant lymphomas and histiocytosis are well described in their respective chapters. As the chapter on pathology points out, the majority of cutaneous tumours in children are benign. It is not surprising, therefore, that the bulk of the separate chapter on 'Skin Tumours' deals with benign lesions. In this chapter there are comprehensive lists of all the skin tumours found in children. The relationship between cellular naevi and malignancy is well described, and the more rare cancers of the skin—melanoma, basal cell naevoid syndrome and xeroderma pigmentosum are adequately covered.

This book is a useful practical guide for doctors working in the more general area of cancer, and should also be useful for those interested in paediatric dermatology.

J.EVERALL

Dermatophilus infection in Animals and Man Edited by D.H.LLOYD and K.C.SELLERS (1976) London: Academic Press. Pp. 322+xviii. Price £7.00.

Whilst the claim that this book is *essential* reading for many in human dermatology may be hard to sustain, there is no doubt that it will interest and stimulate. Dermatophilus infection in cattle (often known as streptothricosis) is characterized by 'an acute or chronic, local or progressive and sometimes fatal exudative dermatitis'. Even those animals which do not die may lose condition and at slaughter the hide may prove unacceptable for tanning. Dermatophilus infection therefore causes economic loss to farmers, most particularly in the tropics, though the disease is also recognized in temperate areas. Cattle are not the only hosts, since sheep, deer, horses, goats, non-human primates and man himself may be infected. Kaplan reviews the disease in humans and although infection transmitted directly from animals is well attested he urges caution in accepting pitted keratolysis of the feet in man as a manifestation of dermatophilus infection. Nevertheless, Kaplan believes that dermatophilus infection is under-diagnosed in man and that further investigation of its role in human disease is warranted.

The causative organism, *Dermatophilus congolensis,* is a filament-forming bacterium which may show flagellated motile 'zoospores' or coccoid forms and there is a distinct 'life cycle' which may, however, be omitted in artificial culture. Under medical laboratory growth conditions colonies of *D. congolensis* may resemble those of *Staphylococcus aureus. D. congolensis* is classed in the Actinomycetales. Antibacterial drugs are effective but antifungal drugs ineffective in treatment.

This book is a record of a symposium on dermatophilus infection held in Nigeria. Much of the repetition usually associated with a book-of-the-symposium has been skilfully edited out to leave a series of papers which tell fairly consecutive stories. It is a credit to its editors and will be enjoyed by all who read it.

W.C.NOBLE

British Journal of Dermatology (1976) **95**, 335.

Review

Adverse reaction to the β-adrenergic blocking drugs

The last 10 years have seen a rapid increase in the use of drugs which inhibit adrenergic *β* receptors, mainly in the cardio-vascular field. The original drug in this field was pronethanol. The use of this drug was limited, however, by unpleasant side effects in man which induced light-headedness and slight inco-ordination followed by nausea and vomiting, and in animals, acute central nervous system toxicity occurred (Black *et al.*, 1964). Chronic toxicity studies showed that thymic tumours developed and later generalized lymphosarcomas and reticulum cell sarcomas (Paget, 1963). Fortunately this drug was replaced by propranolol (1-isopropylamino-3 (1-naphthyloxy)-2-propranolol hydrochloride).

Propranolol was shown to be about 10 times more effective than pronethanol in its *β* blocking activity, whilst the LD 50 in mice was about the same. No evidence of tumour formation was found (Black *et al.*, 1964). This drug is now extensively used for the control of cardiac arrhythmias, angina pectoris, and to a lesser extent hypertension. Propranolol is unfortunately an equally effective inhibitor of *β* receptors in the bronchial tree and the peripheral circulation. The search therefore started for drugs with blocking activity only on cardiac *β* receptors.

The first of these drugs to gain acceptance was practolol (4-(2-hydroxy-3-isopropylamine propoxy) acetanilide). The structure of practolol is compared with propranolol and noradrenaline in Fig. 1. The substitution of the acetamide group in the p-position greatly alters the physico-chemical properties of the compound in comparison with propranolol. Lipid solubility is considerably reduced resulting in very little of the drug entering the CNS and approximately 90% being excreted unchanged in the urine.

The acute toxicity is at least twice that of propranolol in rodents but no subacute or chronic effects have been noticed in animals (Barrett, 1971). Practolol has now become widely used in situations where *β* blockade was necessary but where propranolol might embarrass the respiratory function. It has also proved very useful in controlling supraventricular tachycardias after myocardial infarction and cardiac surgery since it does not have the deleterious haemodynamic effect of propranolol.

Adverse reactions

These drugs began to cause concern when Rowland & Stevenson (1972) reported exfoliative dermatitis in a patient which they attributed to practolol. In an earlier report Wiseman (1971) had described seven patients in a survey of 2100 who had developed micropapular, eczematous or urticarial reactions whilst taking practolol. Assem & Banks (1973) reported a psoriasiform and lichenoid skin eruption in a patient on practolol. This patient was also shown to develop ANF and LE cells and lymphocyte transformation occurred in response to practolol *in vitro*. Raftery & Denman (1973) described patients who developed an LE syndrome whilst on practolol. This consisted of arthralgia particularly of the small joints of the hands, a rash, fever, a raised ESR, and positive LE cells and ANF. Antibody to DNA was not demonstrated, a finding which Hughes (1971) states is typical of drug-induced LE syndromes.

Felix, Ive & Dahl (1974) then reported a series of twenty-one patients suffering from practolol-induced rashes which they felt fitted into a clinically recognizable diagnostic picture. They described this as a psoriasiform eruption most marked over the bony prominences, invariably preceded by hyperkeratotic changes of the palms and soles and sides of the fingers, followed by an erythematous, scaling, marginated rash involving other areas of the body. The rash tended to develop slowly over

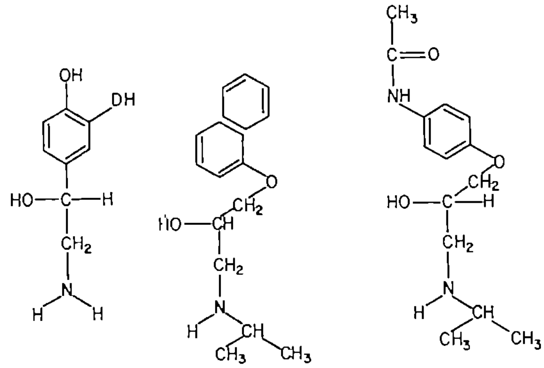

Noradrenaline Propranolol Practolol

FIGURE I

several months. The histological changes were thought to be unlike psoriasis and consisted of patchy swelling and disruption of the basement membrane with epidermal atrophy and colloid bodies migrating through the epidermis.

Wright (1975) reported a series of twenty-seven patients who developed damage to the conjunctiva, on occasions leading to severe impairment of vision. The main findings were of a reduction in tear flow followed by corneal changes. Firstly hyperaemia appeared with increased prominence of the papillary tufts; a less ordered pattern of blood vessels later developed with some areas of relative avascularity and other areas where vessels actively proliferated. This was followed by a subconjunctival sheet of fibrovascular tissue which later contracted to produce the typical scarring, conjunctival shrinkage, and fornix obliteration. In the most severely affected patients unusual corneal changes were seen consisting of the sudden appearances of yellow or white opacities involving the whole thickness of the stroma. Alternatively sudden stromal melting and loss of corneal substance lead to rapid perforation. Nineteen of these patients also had had skin rashes at some time which essentially fitted with those described by Felix *et al.* (1974) but Wright felt that the eye signs were easily distinguished from those associated with other skin conditions (personal communication). Fifteen of Wright's patients also complained of symptoms associated with hearing and five required surgery for secretory otitis media. Five patients suffered from oral or nasal ulceration but none had the classical xerostoma of Sjögrens syndrome.

Brown *et al.* (1974) reported three cases of sclerosing peritonitis in patients on practolol and since then further cases have been recorded (Windsor, Kurrein & Dyer, 1975; Hansen *et al.*, 1975). Essentially these patients present with non-specific abdominal signs and symptoms and usually a mass is felt. At laparotomy the peritoneal cavity appears obliterated with gross thickening and contraction of the parietal and visceral layers. The small bowel is encased in a rigid tube of thickened peritoneum and is considerably shortened. Histology of the peritoneal biopsy shows a non-specific inflammatory reaction. Beneath the attenuated mesothelium is a thick layer of laminated fibrous tissue. There is no evidence of giant cells or panniculitis. This condition can develop several months after stopping the drug.

One patient has been described who developed the nephrotic syndrome (Farr, Wingate & Shaw, 1975) whilst on practolol but electron microscopy and immunofluorescence were not done on the renal biopsy.

There have been sporadic reports of similar occurrences with other β blockers. Several cases have been referred to Mr P.Wright as having the eye syndrome whilst on propranolol but they have all turned out to have eye problems not related to drug ingestion (personal communication). From our present evidence propranolol seems to be safe from the point of view of these adverse reactions and as yet no convincing evidence has accumulated to implicate any of the other β blocking drugs, though a careful watch must be maintained and all suspected cases thoroughly investigated.

The mechanism of the practolol reaction

The adverse reactions mentioned above do to a certain extent form a syndrome, in that a patient with one of these adverse effects is reasonably likely to have others. Thus it would seem necessary to develop a unified explanation.

Several types of investigation have been carried out on these patients. Raftery & Denman (1973) demonstrated that their three patients with an SLE syndrome had LE cells and circulating antinuclear antibody but did not have anti-DNA antibodies. They were unable to show specific immunological sensitization to practolol by the lymphocytes nor were they able to show antibody receptors for practolol using the rosette technique described by Perrudet-Badoux & Frei (1971). However, they claimed that a depression in the lymphocyte transformation to PHA, Con A, and Pokeweed mitogen occurred. They suggested that lymphocyte function may be capable of alteration by drugs which have receptor blocking functions and hypothesised that practolol is capable of suppressing a 'T' cell subpopulation which controls the emergence of 'B' lymphocytes, with auto-immune propensities.

Further evidence for the development of auto-antibodies came from the investigations of Amos, Brigden & McKerron (1975) into the patient described by Wright (1975) with the oculomucocutaneous syndrome. They described a circulating antibody which binds to the intercellular region of guinea-pig stratified epithelium which appeared similar in its site of binding and in titre to the antibodies seen in pemphigus. They were, however, able to distinguish between the two groups by their binding ability to trypsin-isolated guinea-pig epidermal cells. The pemphigus antibodies bound to the membranes of these cells whereas the practolol antibodies did not. These patients also had circulating antinuclear antibodies.

Sixteen of these patients have been further investigated for their lymphocyte responses and all had normal transformation to PHA. Only one of nine tested was shown to transform to practolol or a practolol-albumin conjugate (Brigden, Amos & Camps, 1976).

In vivo binding of intercellular antibody was seen in three patients from whom satisfactory conjunctival biopsies were obtained. Felix *et al.* (1974) described IgG, IgM, and C_3 deposition in the region of the dermo–epidermal junction, though they did not look for circulating antibodies. In one patient from whom post mortem material was available who had high titres of circulating intercellular antibody, no *in vivo* binding was demonstrated though there was IgG deposition at the dermo–epidermal junction. Unfortunately the material was not suitable for elution studies. A recent case reported by van Joost, Crone & Overdijk (1976) which probably fits into Wright's oculomucocutaneous syndrome was also found to have IgG and complement deposition along the basement membrane of the buccal mucosa.

There seems to be little doubt that immunological mechanisms are involved in the practolol syndrome, however it is far from clear in what way this might be mediated. There is only rather limited evidence that these patients have become sensitized in the immunological sense to practolol—three patients with positive skin tests (Felix *et al.*, 1974), the suggestion by Assem (1974) that lymphocytes could be stimulated in three patients on practolol and two patients on propranolol by addition of the drugs *in vitro*, and the propranolol linked antibodies seen in one patient who developed agranulocytosis (Nawaki & Ritz, 1973).

An alternative and attractive hypothesis suggests that practolol has a direct pharmacological effect on the control of the immune response, possibly affecting the 'T' lymphocyte control of 'B' lymphocytes with the ability to produce auto-antibodies (Raftery & Denman, 1973). This hypothesis is supported by the evidence of the development of two distinct auto-antibodies, the antinuclear and intercellular antibodies, in many of these patients. This hypothesis would have to explain why it is apparently only practolol that is capable of producing this effect and why it only affects a relatively small number of patients receiving this drug. It may be that the postulated lymphocyte receptors involved in any pharmacological control of lymphocyte response differ in different individuals and that some individuals have receptors capable of being blocked by practolol. Gaylarde & Sarkany (1975) suggest that the mechanism of the psoriariform eruption may be due to β blockade reducing epidermal cyclic AMP leading to cell division but this phenomenon must also take into account the idiosyncratic nature of the response. Furthermore there is no evidence that epidermal cell turnover is increased.

Practolol is now being steadily withdrawn from use for long term therapy so we are unlikely to see much more clinical material. However, this is an extremely important field for further investigation as it is important to avoid running into these problems with new and more selective β blocking drugs. There is also a possibility that these drugs may prove useful in the pharmacological control of the immune response.

MRC Clinical Research Fellow, W.D.BRIGDEN
Westminster Hospital

Enfield Group of Hospitals J.ALMEYDA

REFERENCES

AMOS, H.E., BRIGDEN, W.D. & MCKERRON, R.A. (1975) Untoward effects associated with practolol: demonstration of antibody binding to epithelial tissue. *British Medical Journal*, **i**, 598.

ASSEM, E.S.K. (1975) Specific immunological responses to propranolol and practolol in man. *British Journal of Clinical Pharmacology*, **2**, 184.

ASSEM, E.S.K. & BANKS, R.A. (1973) Practolol induced drug eruption. *Proceedings of the Royal Society of Medicine*, **66**, 179.

BARRETT, A.M. (1971) The pharmacology of practolol. *Postgraduate Medical Journal*. Suppl., **47**, 7.

BLACK, J.W., CROWTHER, A.F., SHANKS, R.G., SMITH, L.H. & DOMHURST, A.C. (1964) A new adrenergic beta-receptor antagonist. *Lancet*, **i**, 1080.

BRIGDEN, W.D., AMOS, H.E. & CAMPS, M. (1976) In press.

BROWN, P., BADDELEY, H., READ, A.E., DAVIES, J.D. & MCGARRY, J. (1974) Sclerosing peritonitis, an unusual reaction to a β adrenergic blocking drug (Practolol). *Lancet*, **ii**, 1477.

FARR, M.J., WINGATE, J.P. & SHAW, J.N. (1975) Practolol and the Nephrotic syndrome. *British Medical Journal*, **ii**, 68.

FELIX, R.H., IVE, F.A. & DAHL, M.G.C. (1974) Cutaneous and ocular reactions to practolol. *British Medical Journal*, **iv**, 321.

GAYLARDE, D.M. & SARKANY, T. (1975) Side effects of practolol. *British Medical Journal*, **iii**, 435.

HANSEN, A., RHEMNEO, P.E.R. & OBERIUS KAPTEJIN, J. (1975) Sclerosing peritonitis and Practolol, and other letters. *Lancet*, **i**, 275.

HUGHES, G.R.V. (1971) Significance of anti-DNA antibodies in systemic lupus erythematosus. *Lancet*, **ii**, 861.

VAN JOOST, TH., CRONE, R.A. & OVERDIJK, A.D. (1976) Ocular cicatricial pemphigoid associated with practolol therapy. *British Journal of Dermatology*, **94**, 447.

NAWAKI, I.V. & RITZ, N.D. (1973) Agranulocytosis due to Propranolol. *Journal of the American Medical Association*, **223**, 1376.

PAGET, G.E. (1963) Carcinogenic action of pronethalol. *British Medical Journal*, **iv**, 1226.

PERRUDET-BADOUX, A. & FREI, P.C. (1971) The detection of antibodies on Blood lymphocytes in Drug Hypersensitivity using a Rosette technique. *International Archives of Allergy and Applied Immunology*, **41**, 149.

RAFTERY, E.B. & DENMAN, A.M. (1973) Systemic lupus erythematosus syndrome induced by Practolol. *British Medical Journal*, **ii**, 452.

ROWLAND, M.G.M. & STEVENSON, C.J. (1972) Exfoliative dermatitis and Practolol. *Lancet*, **i**, 1130.

WINDSOR, W.O., KURREIN, F. & DYER, N.H. (1975) Fibrinous peritonitis, a complication of Practolol therapy. *British Medical Journal*, **ii**, 68.

WISEMAN, R.A. (1971) Practolol-accumulated data on unwanted side effects. *Postgraduate Medical Journal*, Suppl. **47**, 68.

WRIGHT, P. (1975) Untoward effects associated with Practolol administration: Oculomucocutaneous syndrome. *British Medical Journal*, **i**, 595.

British Journal of Dermatology (1976) 95, 339.

News and Notices

The British Association of Dermatologists

Applications are invited for the Dowling Travelling Fellowship which is open to registered medical practitioners who are British graduates and who hold a higher medical qualification. The aim of the Fellowship is to encourage merit in junior physicians working in the field of Dermatology before they become established in senior academic and professional posts.

Candidates must have a suitable training and experience in clinical dermatology, in laboratory medicine and in research methods; they will normally hold an appointment in a dermatological unit or department of a Teaching Hospital in the United Kingdom. They are required to furnish an essay on the nature of the work to be carried out and indicate at what centres the necessary facilities could be provided.

The Fellowship, which is normally awarded every third year, will be tenable for one academic year and will be of value up to £4,000. There is no bar to the successful Fellow seeking other monies to supplement this award if deemed necessary.

Enquiries or applications which should be submitted before 31 January 1977 should be made to the Hon. Secretary, Dr K.V. Sanderson, 1 Wimpole Street, London, W1M 8AE.

European Society for Dermatological Research, Poster Award

The ESDR Poster Award for the best poster presentation at the annual ESDR meeting, kindly offered by Max Ritter, Pharmaceutical Specialities, Zürich/CH, has been given in 1976 to H. Hönigsmann, G. Stingl, K. Holubar, E. Wolff-Schreiner, K. Konrad & K. Wolff, Division of Experimental Dermatology, Department of Dermatology I, University of Vienna, for the poster 'Autoantibodies and immune complexes in immunodermatoses. Mapping of fine structural binding sites'.

APPOINTMENTS

South East Thames Regional Health Authority

Dr Keith Liddell has been appointed Consultant Dermatologist to the South East Thames Regional Health Authority, an area which covers Eastbourne and Hastings. Dr Liddell graduated M.B., Ch.B., from Leeds University in 1967 and after a year in General Medicine he became Registrar in Dermatology at the Leeds General Infirmary. Recently he has been Senior Registrar in Dermatology at the Southampton University Hospital Group. He obtained his M.R.C.P. (U.K.) in 1973 and was awarded a Nuffield Travelling Scholarship in India in 1966. He has published a number of clinical papers in Dermatology and his main interests are Epidemology of skin diseases, acne and skin markers of internal disease.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Aldenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm³	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: Beare, J.M. & Wilson Jones, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 95, Number 3, September 1976

CONTENTS

Clinical and Laboratory Investigations

PAGE

Epithelioma Adenoides Cysticum: Genetic Update 225
DAVID E.ANDERSON AND JAMES B.HOWELL

Transformation of Epithelioma Adenoides Cysticum into Multiple Rodent Ulcers: Fact or Fallacy. A Historical Vignette 233
J.B.HOWELL AND DAVID E.ANDERSON

Histopathological Spectrum of Erythema Multiforme 243
T.R.BEDI AND HERMANN PINKUS

Histology Simulating Reticulosis in Secondary Syphilis 251
REBECCA E.I.COCHRAN, JOHN THOMSON, K.A.FLEMING AND ALEXANDRA M.M.STRONG

Changes in Transepidermal Water Loss and the Composition of Epidermal Lecithin after Applications of Pure Fatty Acid Triglycerides to the Skin of Essential Fatty Acid-deficient Rats 255
P.J.HARTOP AND C.PROTTEY

The Diffusion of Nandrolone through Hydrated Human Cadaver Skin 265
M.I.FOREMAN AND I.KELLY

Lysosomal Hydrolases of the Epidermis. 6. Changes in Disease 271
P.D.MIER AND JOSÉ J.M.A. VAN DEN HURK

The Fate of Staphylococcal Exfoliatin in Newborn and Adult Mice 275
PETER FRITSCH, PETER ELIAS AND JANOS VARGA

Hypersensitivity to Bacteria in Eczema. II. Titre and Immunoglobulin Class of Antibodies to Staphylococci and Micrococci 285
W.E.PARISH, ESTHER WELBOURN AND R.H.CHAMPION

Immunological Aspects of Psoriasis. II. Dissociated Impairment of Thymus-Dependent Lymphocytes 295
J.J.GUILHOU, J.MEYNADIER, J.CLOT, E.CHARMASSON, M.DARDENNE AND J.BROCHIER

Alternative Pathway Complement Activation: A Possible Mechanism Inducing Skin Lesions in Benign Gonococcal Sepsis 303
REINER SCHERER AND OTTO BRAUN-FALCO

The Effect of Long Term Tetracycline Treatment for Acne Vulgaris on the Occurrence of R Factors in the Intestinal Flora of Man 311
M.V.VALTONEN, V.V.VALTONEN, O.P.SALO AND P.H.MÄKELÄ

Pharmacology and Treatment

A Double-blind Trial of Clomocycline in the Treatment of Persistent Palmoplantar Pustulosis 317
JOANNA M.WARD, MARGARET F.CORBETT AND M.J.HANNA

Co-operative Double-blind Trial of an Antibiotic/Corticoid Combination in Impetiginized Atopic Dermatitis 323
GERALD N.WACHS AND HOWARD I.MAIBACH

Brief Communications

Corticotrophin and Vitiligo (Preliminary Observations) 329
B.B.GOKHALE AND TARA B.GOKHALE

Muscle Herniation of the Lower Legs 329
JULIAN VERBOV

Canadian-Irish Dermatological Meeting 330

Book Reviews 332

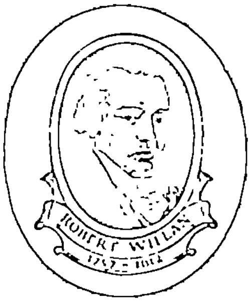
Review

Adverse Reaction to the β-adrenergic Blocking Drugs 335
W.D.BRIGDEN AND J.ALMEYDA

News and Notices 339

Hee
11.12.76

Volume 95, Number 4, October 1976



British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

British Journal of Dermatology

EDITOR

DR R. H. CHAMPION

Addenbrooke's Hospital, Hills Road Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

PROF. J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London

DR RONALD MARKS, Cardiff

DR P.D.MIER, Nijmegen

DR J.N.S.MITCHELL, Royal Air Force

DR W.C.NOBLE, London

DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS

DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed and bound in Great Britain at the Alden Press, Oxford

IMPORTANT NEW PRODUCT FOR PSORIASIS

Dithranol
olan

Much easier to apply, far easier to remove, and actually more effective.

DITHROLAN is quite simply the most convenient and effective form of dithranol in the treatment of psoriasis.

A careful and painstaking study carried out over the last 5 years at the Beaumont Hospital, Lancaster, demonstrated that a newly developed formulation of dithranol, now introduced as DITHROLAN, was not only very much easier to use than traditional Dithranol Paste, but was actually more effective[1,2].

DITHROLAN adheres easily to the skin and can be applied quickly and conveniently. Previous applications of DITHROLAN can be removed simply by bathing – obviating the need for oil. As a result, patients actually carry out their treatment.

Dermatologists will appreciate DITHROLAN because of the considerable saving in treatment effort and time, and because of the excellent results obtained.

Presentation: DITHROLAN – containing 0·5% Dithranol B.P, 0·5% Salicylic Acid B.P. 90 gram amber glass jar. PL 0173/0024

References: 1. Brit. J. Derm. **93**, *205, 1975. 2. Dermatology Digest* Feb, *20, 1976.*

Further information and literature available from:

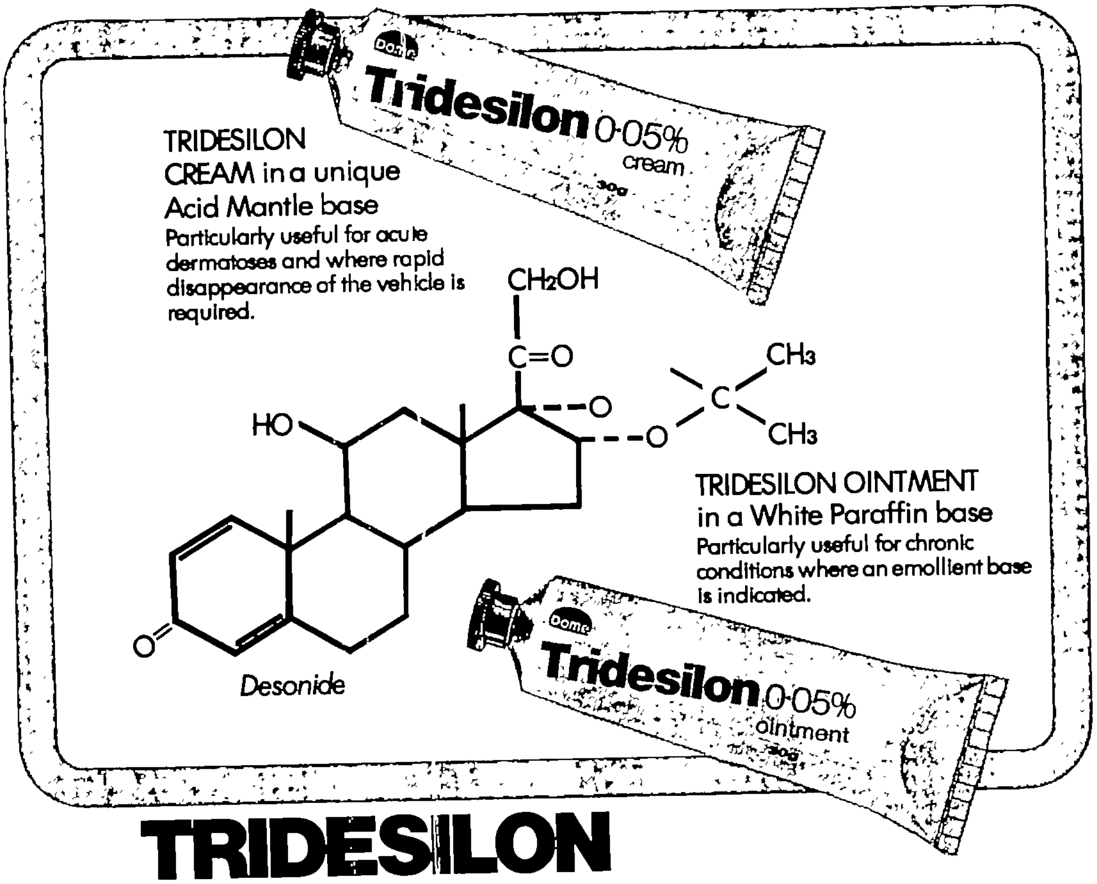
Dermal Laboratories Limited,
Tatmore Place, Gosmore, Hitchin,
Herts, SG4 7QR, England

Dithrolan

non-fluorinated

TRIDESILON

offers a choice



TRIDESILON
CREAM in a unique
Acid Mantle base
Particularly useful for acute dermatoses and where rapid disappearance of the vehicle is required.

Desonide

TRIDESILON OINTMENT
in a White Paraffin base
Particularly useful for chronic conditions where an emollient base is indicated.

TRIDESILON

the effective, non-fluorinated topical steroid

Full prescribing information available

Dome Laboratories
Division of Miles Laboratories Ltd
Stoke Court Stoke Poges Slough SL2 4LY
Tel Farnham Common 2151

TRIDESILON is a trade mark DM 682/A

'To lie in the soothing embrace of a comfortable warm bath while a medically effective emollient performs its healing task...'

Extract from the recording "The Dry Skin"

Oilatum Emollient

gentle restorer of dry skin

Packs: 65ml and 150ml bottles; 1 litre dispensing pack
Oilatum Emollient is classified as a
drug and is prescribable on EC10 without restriction.

If you have not heard the record 'A Refresher Course in Dermatology for General Practitioners' – a 15 minute lecture by a Consultant Dermatologist – we shall be pleased to send you a copy. Samples of Oilatum Emollient are available.

New pack
65ml

Full information on request

Stiefel Laboratories (UK) Limited
825 Yeovil Road, Slough SL1 4JA

84%

# of plantar warts wiped out with Salactol.

Salactol is the most reliable, best documented form of treatment yet developed for warts. The evidence for Salactol is impressive. In a double blind controlled trial [1] 84% of cases with plantar warts were cured within a 12 week period. Its value is further confirmed in papers [2, 3] *where Salactol is actually recommended by name.*

And it's a British product through and through.

Reliability, convenience and efficacy make Salactol the most widely accepted preparation for the treatment of warts in the UK.

Full information on Salactol is available from:

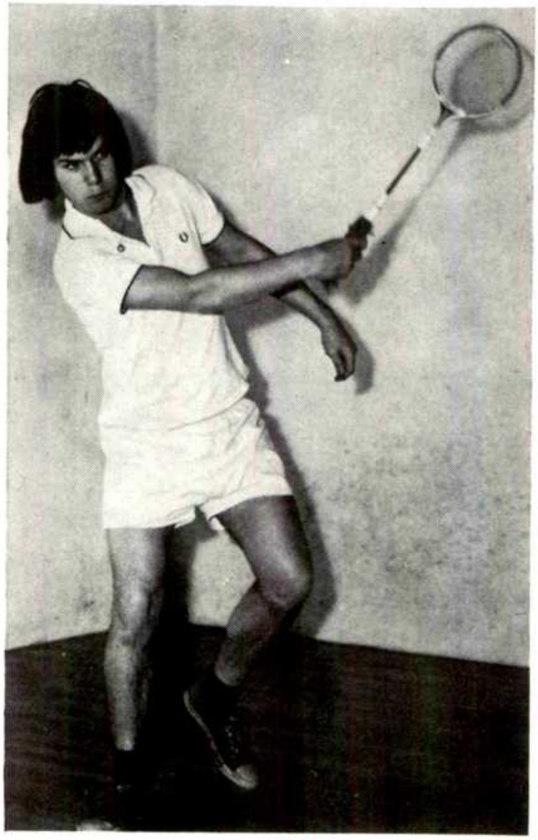
Dermal Laboratories Limited
Tatmore Place, Gosmore, Hitchin
Hertfordshire SG4 7QR

Salactol contains salicylic acid and lactic acid in a flexible collodion base and is presented in a 10ml bottle (more than enough for a full course of treatment) with a special applicator.

References:

1. Bunney, Mary H. (1971), Practitioner, **207**, 197.
2. Pegum, J. S. (1972), Practitioner, **209**, 453.
3. Bunney, Mary H. (1974), Prescribers Journal, **14**, 118.

ACNE?



prescribe patient confidence

"Acne is an almost universal complaint in adolescence."[1]

"Many patients when they seek the help of their doctor are depressed, discouraged and disillusioned. Their own efforts at treatment have failed, or the first prescription was not the cure they anticipated. In the management of acne the patient must appreciate that he or she may have to persevere with treatment throughout their teens, and it should be emphasised that in this way one hopes to limit as far as possible the severity of the lesions and prevent severe or permanent scarring."[2]

When patients like these ask for your help remember that clinical reports show that acne responds to Quinoderm.

Available as Quinoderm
with **Hydrocortisone Cream 30G.** for initial treatment.
Quinoderm Cream 25G. for subsequent treatment.

1. 2. Practitioner 1970, 204, 639

Quinoderm Hydrocortisone 30G.
Benzoyl Peroxide 10.0%
Potassium Hydroxyquinoline,
Sulphate 0.5%
Hydrocortisone 1%
in an astringent cream base.

Quinoderm Cream 25G.
Benzoyl Peroxide 10.0%
Potassium Hydroxyquinoline
Sulphate 0.5%
in an astringent cream base.

Quinoderm

For further information and literature please write to THE QUINODERM COMPANY, OLDHAM, LANCS.

PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS

the modern basis of treatment

'. . . to restrict topical steroid therapy to short periods for getting the disease under control and then to change to tar preparations which may prevent rebound exacerbations.'

Practitioner, 1970, **204**, 625.

New Alphosyl HC cream

for short-term control

The unique descaling, healing and anti-inflammatory properties of Alphosyl, plus the added anti-inflammatory action of hydrocortisone to get the disease swiftly under control.

Alphosyl cream

lotion and shampoo for long-term maintenance

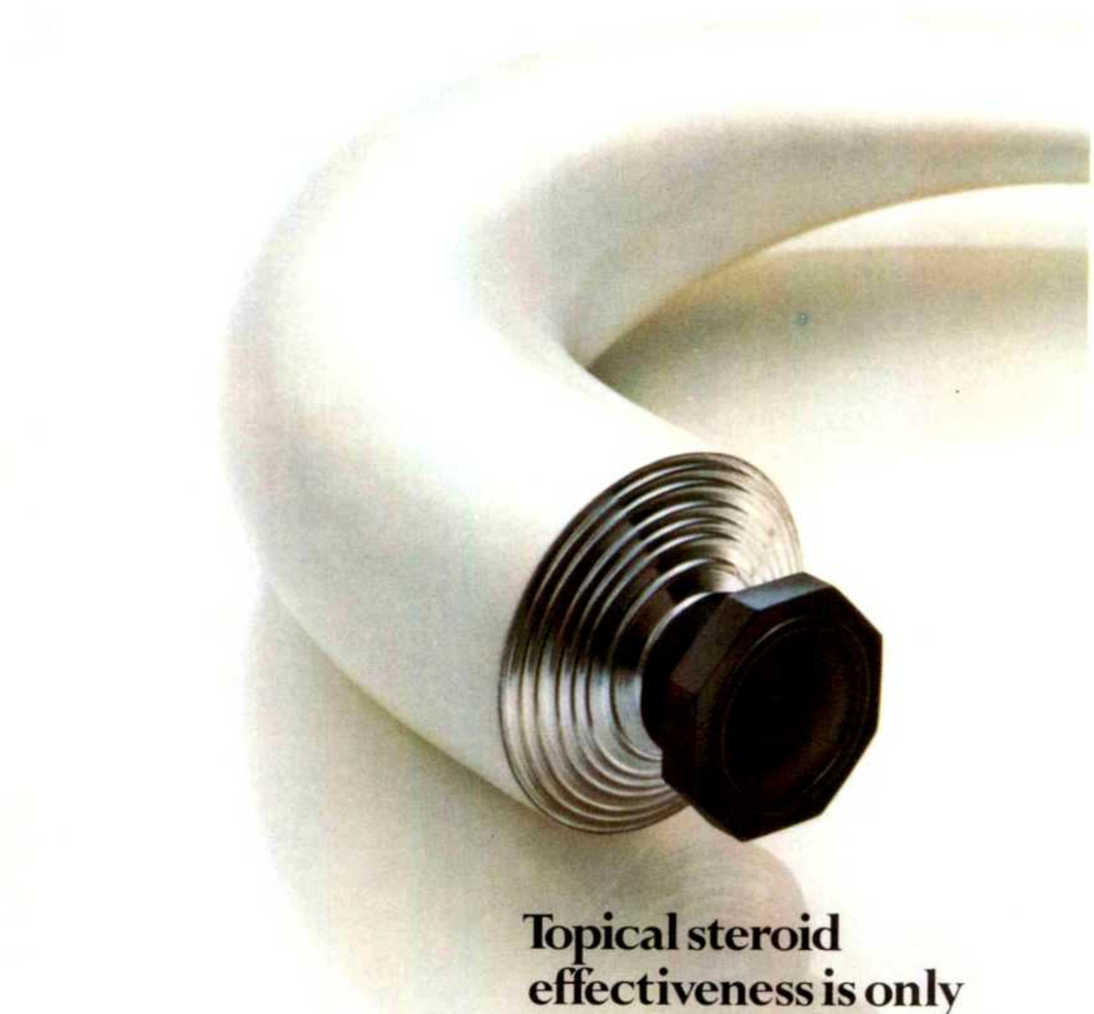
'. . . it (Alphosyl) does not have the disadvantage of staining, burning and discolouration that occur with other topical preparations.' Br.med.J., 1967, **4**, 535.

Alphosyl

a whole range of treatment for psoriasis

Alphosyl cream, lotion and shampoo contain a highly refined extract of coal tar and allantoin. Alphosyl HC in addition contains 0.5% hydrocortisone. Please send to us for samples, clinical information and Data Sheets.

Stafford-Miller The Professional Relations Division, Stafford-Miller Limited, Hatfield, Herts.



Topical steroid effectiveness is only half the Locoid story

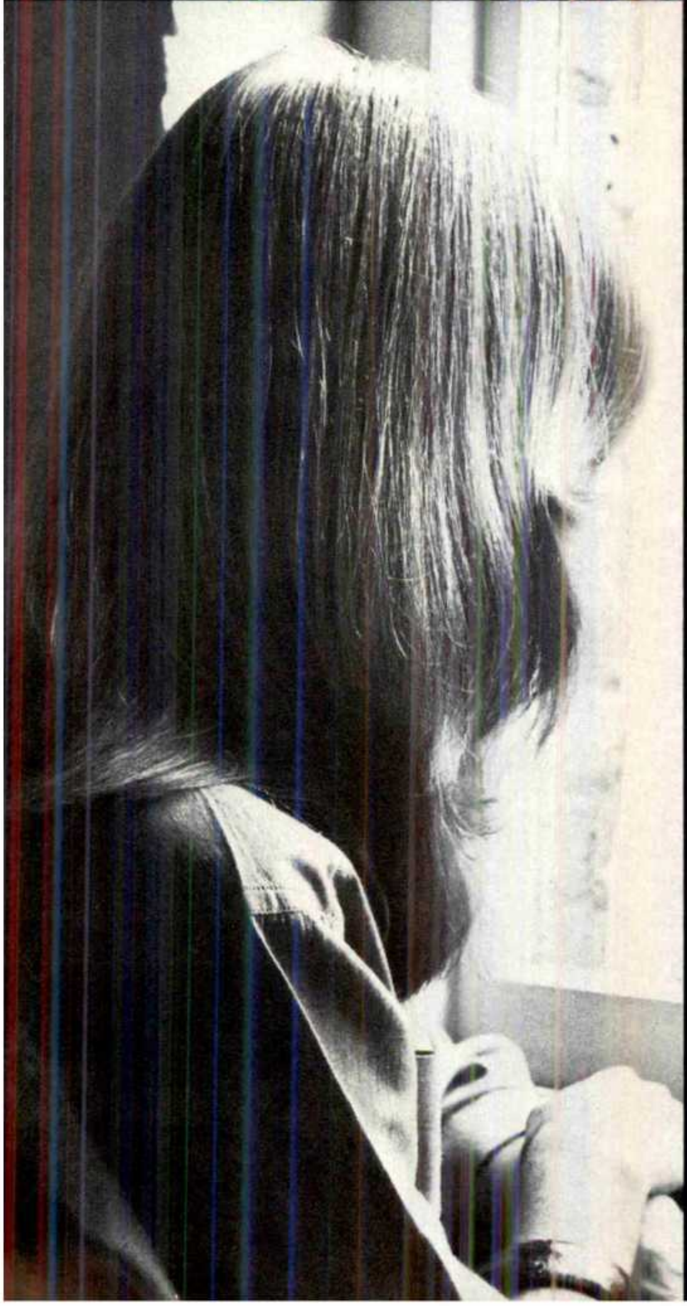
...the other half is the relative freedom from topical side effects which are often encountered with the fluorinated steroids. Locoid offers the same level of success as these potent preparations – without fluorination.

locoid

the non-fluorinated way to topical steroid success

Locoid is a trade mark for hydrocortisone 17-butyrate.
Full information available on request:
Brocades (Great Britain) Ltd.,
Brocades House, West Byfleet, Surrey, KT14 6RA.
Telephone: Byfleet 45536.

She's been to the chemist she's been to her doctor now she's come to you



In acne "... not all patients respond satisfactorily [to tetracycline] and there is a need for more effective or alternative therapy."

SEPTRIN* "... has been clinically effective in patients who did not respond to long-term tetracycline."

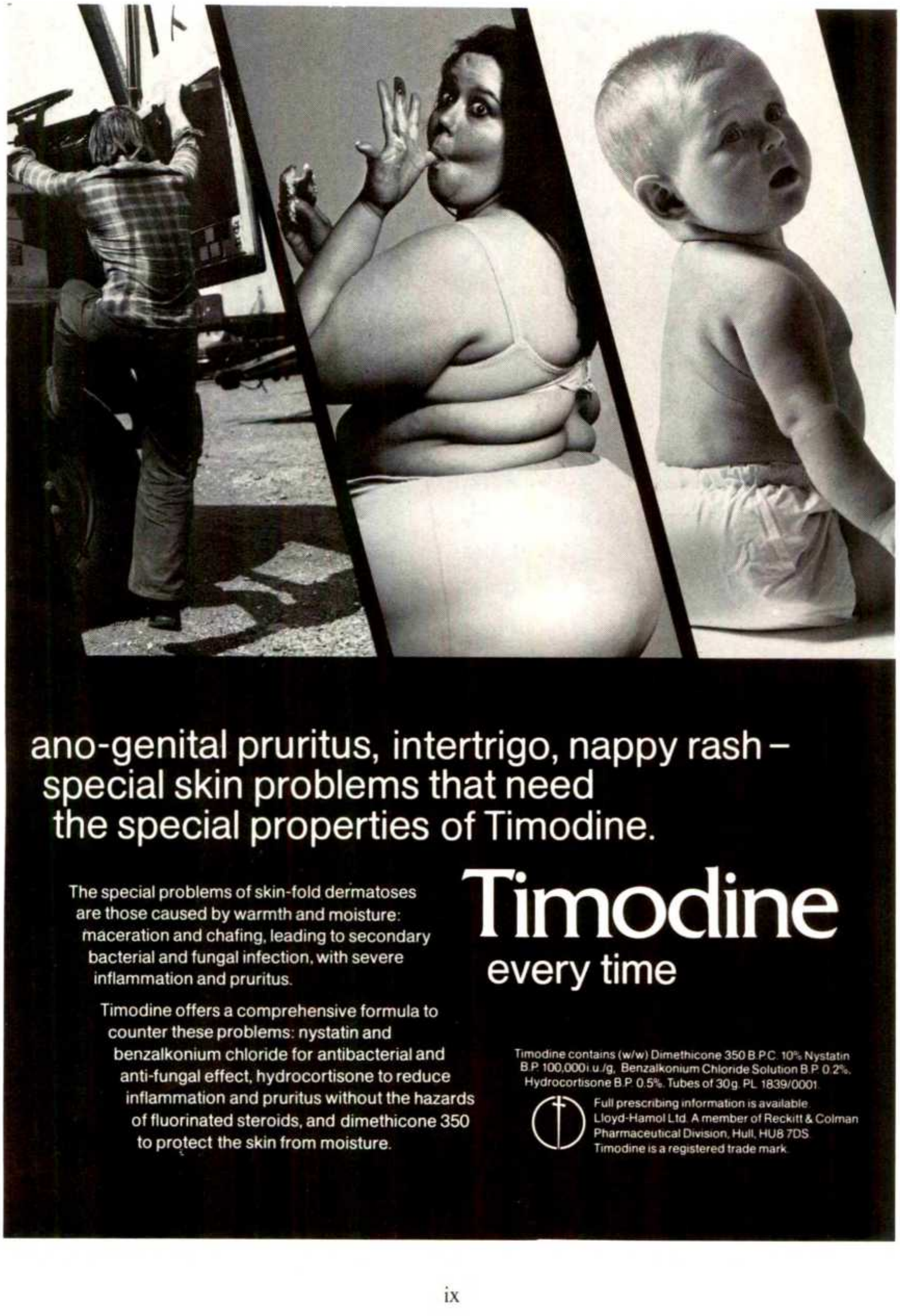
Brit J Derm, (1971), **84**, 366.

SEPTRIN FOR ACNE

Wellcome

Septrin contains trimethoprim and sulphamethoxazole. Full prescribing information is available on request.
Wellcome Medical Division
The Wellcome Foundation Ltd.
Berkhamsted, Herts

*Trade Mark



ano-genital pruritus, intertrigo, nappy rash –
special skin problems that need
the special properties of Timodine.

The special problems of skin-fold dermatoses are those caused by warmth and moisture: maceration and chafing, leading to secondary bacterial and fungal infection, with severe inflammation and pruritus.

Timodine offers a comprehensive formula to counter these problems: nystatin and benzalkonium chloride for antibacterial and anti-fungal effect, hydrocortisone to reduce inflammation and pruritus without the hazards of fluorinated steroids, and dimethicone 350 to protect the skin from moisture.

Timodine
every time

Timodine contains (w/w) Dimethicone 350 B.P.C. 10% Nystatin B.P. 100,000i.u./g, Benzalkonium Chloride Solution B.P. 0.2%, Hydrocortisone B.P. 0.5%. Tubes of 30g. PL 1839/0001.

Full prescribing information is available.
Lloyd-Hamol Ltd. A member of Reckitt & Colman
Pharmaceutical Division, Hull, HU8 7DS.
Timodine is a registered trade mark.

Canesten®
clotrimazole
broad-spectrum antifungal

More and more doctors are finding Canesten a highly effective and trouble-free treatment for all the fungal infections commonly seen in hospital practice.

In Gynaecology:
6-day Canesten
the only effective 6-day treatment for candida and mixed vaginal infections.

In Dermatology:
broad-spectrum Canesten
now the most widely prescribed treatment for fungal skin infections.

The new solution for fungal skin infections

New Canesten Solution

As effective as Canesten cream in treating a wide range of fungal skin infections

Especially suitable for applying to sensitive, moist or hairy areas

Also suitable for fungal infections of the outer ear (otitis externa)

Both the cream and the new solution are easy to apply, non-greasy, non-staining and odourless

Bayer

Further information, including data sheet, is available from
Bayer UK Limited, Pharmaceutical Division,
Haywards Heath, West Sussex RH16 1TP
Telephone: Haywards Heath (0444) 57911
® Registered Trade Mark of Bayer Germany

British Journal of Dermatology (1976) **95**, 341.

Clinical and Laboratory Investigations

Practical applications of the Quantimet 720,* an image analysing computer, in the field of investigative dermatology

W.PICTON, H.DEVITT AND M.A.FORGIE

Scientific Development Group, Organon Laboratories Ltd.,
Newhouse, Lanarkshire ML1 5SH, Scotland

Accepted for publication 25 February 1976

SUMMARY

Image analysing computers can perform quantitative measurements of epidermal thickness, sebaceous gland volume, and labelling indices of epidermis easily, rapidly and automatically. All these parameters are extremely important in investigative dermatology and this paper describes how they are measured using the Quantimet 720.

Investigators over the years have extracted quantitative data from sebaceous gland volume (Ebling, 1957) and mean epidermal thickness (Schellander & Marks, 1973) by time-consuming planimetric methods. The number of cells in DNA synthesis at any one time can be demonstrated by the autoradiographic flash labelling technique. This technique involves counting the cells which have incorporated ^{3}H-thymidine and expressing the result either as a percentage of the total number of basal cells or of the total number of basal and supra-basal cells, or as the number of labelled cells per unit length of interfollicular epidermis. This technique is tedi us, time-consuming and requires at least two or three investigators to examine the sections and perform the measurement. These three measurements are performed easily and rapidly using the Quantimet 720.

MATERIALS AND METHODS

Post mortem human abdominal skin samples were obtained from 5 males (age range 63–77 years). Samples of skin were taken from the dorso-lumbar region of: (1) Hairless hamster (Wright's, Essex)—female, weight range 100–120 g (4–6 months old). (2) Hairless rats (C.S.E.A.L.-C.N.R.S., 45045 Orleans-Cedex, France)—male, weight range 290–340 g (8–10 weeks old). (3) Haired rats (CSH-Wistar)—male, weight range 160–200 g (5–6 weeks old).

All skin samples, 1 × 1 cm square, were placed onto Sleek adhesive tape so that the epidermal surface was fixed flat. Samples were placed in formol saline for 24 h, after which time the adhesive tape could be easily peeled off the rigid flattened skin samples. All samples were processed in the normal histological manner, embedded in paraffin wax and sectioned at a microtome setting of 7 μm. For the labelling index experiments, hairless hamsters were injected intracutaneously with 10 μCi tritiated thymidine (sp. activity = 21 Ci/mmol) in 0·1 ml physiological saline 1 h prior to sacrifice and the

---

* The Quantimet is supplied by Cambridge Instruments, Moat House, Moat Lane, Melbourn, Royston, Herts. SG8 6EJ.

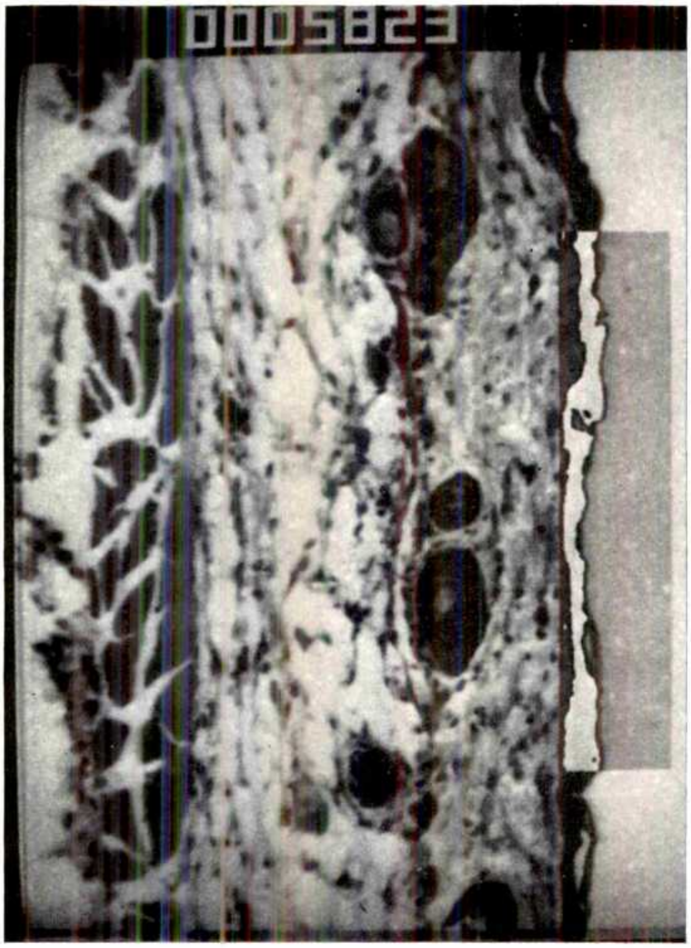


FIGURE 1. Measurements of epidermal area within the set length of the variable frame. The figure in the top left hand corner represents the area in square picture points.

taking of skin samples. Fixation and preparation of skin sections was carried out as described above. Sections were prepared and processed autoradiographically by dipping in Ilford K5 nuclear emulsion. The time of exposure was 3 weeks.

The staining of the skin sections for the three measurements will be described below.

General principles of the Quantimet 720 (for a more detailed general description the reader is referred to Fisher, 1971).

The Quantimet 720 operates in the following way. The image of a skin section is placed before a television camera via a microscope input peripheral. The camera, in one scan, resolves the image into

TABLE 1. Repeated measurements of specified features in one single defined field

For epidermal thickness	For sebaceous gland volume	For labelling index
Repeated measurement of the epidermal area of detected epidermis in a single defined field	Repeated measurement of the cross-sectional area of the same sebaceous gland as drawn by the operator	Repeated count of the labelled cells in a single defined length of epidermis after detection and sizing thresholds have been set
(p.p.^{2})	(p.p.^{2})	
5613	2708	6
5613	2560	6
5614	2524	6
5616	2616	6
5619	2756	6
5611	2632	6
5616	2608	6
5607	2576	6
5605	2544	6
5617	2600	6
5163 ± 2 (10)	2612 ± 24 (10)	6 ± 0 (10)

Mean ± s.e. Figures in parentheses denote the number of estimations.

TABLE 2. Epidermal thickness, sebaceous gland volume and labelling index

Species	Epidermal thickness Mean ± s.e. (μm)	Sebaceous gland volume Mean ± s.e. ($\mu m^3 \times 10^{-3}$)	Labelling index (No. of cells labelled per mm interfollicular epidermis)
Human (63–77 years old)	16·9 ± 1·4 (5)	2681 ± 636 (4)	—
Hairless hamster (4–6 months old)	11·8 ± 0·7 (10)	109·9 ± 4·8 (6)	5·8 ± 0·3 (7)
Hairless rat (8–10 weeks old)	16·3 ± 0·8 (6)	290·4 ± 34·3 (6)	—
Haired rat (6–8 weeks old)	15·1 ± 1·4 (6)	58·4 ± 5·6 (6)	—

Figures in parentheses denote the number of samples in the group.

605,000 picture points (p.p.). Points are represented by digitised video signals on a scale reading of 0 (black) to 64 (white); the readings 1 to 63 being termed grey levels. The coloured image of the skin section is therefore converted to an image which consists of whites, various shades of grey, and blacks. The digitized value of each picture point is stored in a computer whilst the image is displayed on a television screen. Features up to a certain grey level or features with the same grey level value can be detected and displayed on the screen by dialling the level (0–64) on a digiswitch. These areas are superimposed on the original image and are shown as bright areas on the screen. A computer then computes the area, intercept, and perimeter of individual features which have been detected, or gives the total area, total intercept, total perimeter and number of features detected in the whole image. The measurements are given in sq. p.p. for area measurements, p.p. for intercept and perimeter, and numbers for count. The length of 1 p.p. can be converted to mm by the use of a calibrated scale.

A variable frame module superimposes a frame of known length and height on the display screen. Only detected features within this frame will then be measured. The use of this module will be illustrated below. A further module, the image editor, which will be referred to later, is a light pen which can be used to outline and edit features of interest on the display screen.

Epidermal thickness

Sections were stained with haematoxylin and eosin such that there is a strong blue epidermis and a pale pink dermis. Measurements of epidermal thickness were made by measuring the area of interfollicular epidermis within a frame of known length (Fig. 1). The reproducibility of this area measurement can be seen in Table 1 where the area of detected epidermis in one single defined field was measured 10 times. The error is always less than 1% unless very small areas are being measured. The mean thickness of the epidermis was calculated by dividing the area of epidermis measured by the frame length. At least 20 × 1 mm lengths per sample were measured for the estimation of epidermal thickness. A group mean (and its standard error) was calculated for the number of animals in the group (Table 2).

Sebaceous gland volume

Sections were stained with haematoxylin and eosin as for routine histology or simply with toluidine blue. Using the image editor, the outline of the cross-sectional area of sebaceous gland was drawn on the display screen (Fig. 2). This outline is recognized as the perimeter of a feature, and its area can be easily measured and displayed on the screen (Fig. 3). The area which has been computed is exact. The error in this measurement arises from the operator outlining the perimeter of the sebaceous gland. Table 1 gives the area of one sebaceous gland as drawn by the operator ten times. There the error is approximately 1%. Originally the cross-sectional areas of 100 different sebaceous glands were measured to arrive at a mean cross-sectional area. However, it was shown that the number could be reduced to 50 without loss in accuracy of the mean. Therefore 50 cross-sectional areas were measured for each sample. It is important to stress that no gland was ever measured twice since measurements were performed only in every fifteenth section. The average volume of the sebaceous gland for each sample was then calculated using the formula

$$\text{Volume} = (\text{mean area})^{3/2} \times 1{\cdot}382 \text{ (Ebling, 1957).}$$

The mean sebaceous gland volume (and its standard error) was calculated for the group (Table 2).

Labelling index measurements

Autoradiograms of hairless hamster skin were stained in 1% aqueous eosin and differentiated in water such that there was a pale pink epidermis. By the method of injecting tritiated thymidine intradermally and of taking the skin sample at the site of the injection, it has been found that the cells which are

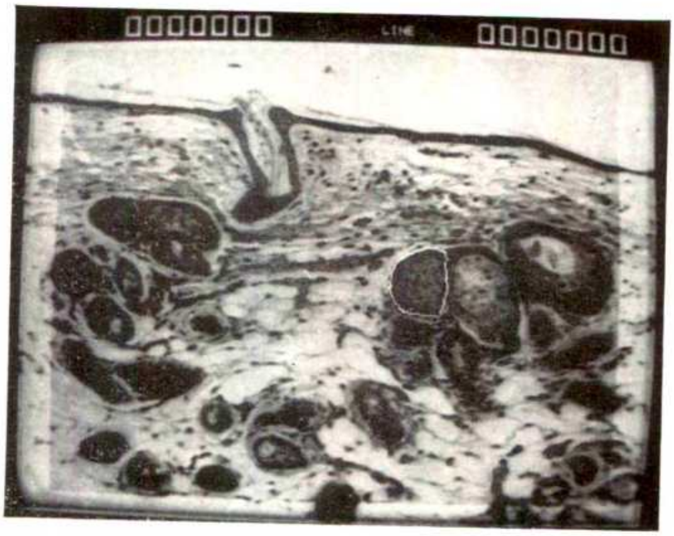


FIGURE 2. Outlining the area of the sebaceous gland to be measured with the 'image editor'.

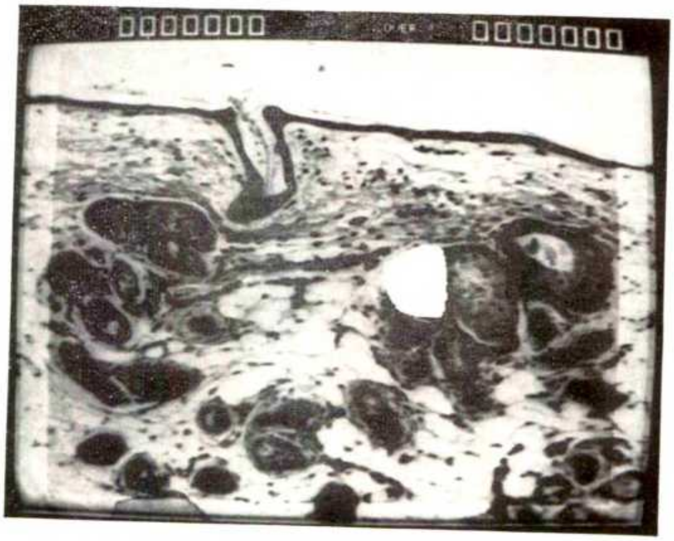


FIGURE 3. Area of the outlined sebaceous gland is displayed on the screen. The area is automatically computed.

synthesizing DNA are very heavily labelled and stand out clearly against the eosin stained sections. For instance in Fig. 4, it is possible to distinguish labelled cells in the hair follicle epithelium and epidermis as distinct black areas over the nucleus, i.e. areas in the emulsion where the silver grains have merged into each other to form definitive areas as shown by detection in the detecting frame in Fig. 4.

At the magnification used in Fig. 4, the projected heights and the projected widths of the detected areas can range from 3 to 9 picture points. By applying a size discriminant of either 1 or 2 picture points, 1 or 2 picture points can be erased from the projected width of the detected areas. This size

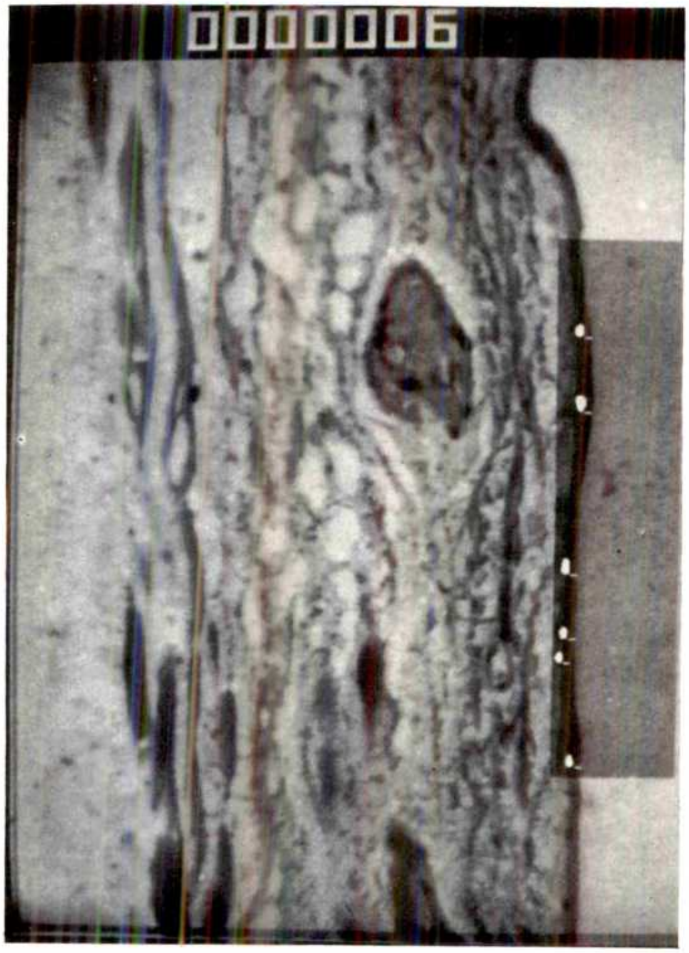


FIGURE 4. Measurement of the number of labelled cells within the set length of the variable frame. The figure in the top left hand corner represents the number of labelled cells in that length of epidermis.

criterion removes the background noise of the television scanner and also removes background silver grains which have been detected, leaving detected areas which represent the labelled cells. The number of detected features is displayed automatically providing a count of labelled cells.

When two labelled cells are in juxtaposition or even overlapping, they are sometimes counted as a single cell. Using the image editor in the 'cut' mode, it is possible for the investigator to draw through the detected area of the two labelled cells and, in doing so, remove a line of detection 1 picture point wide. The Quantimet then counts the cells as two.

Our measurements of labelling index have been confined to hairless hamsters. The interfollicular epidermis of the hairless hamster normally consists of a single cell basal layer upon which resides the stratum corneum. The number of basal cells per 1 mm length of interfollicular epidermis has been counted and is equal to 116 ± 5 (Mean $\pm$ s.e. $n = 10$, range $= 100$–140). The error in the labelling index will stem from the error of cells per 1 mm length of interfollicular epidermis. This is less than 5%.

We consider, therefore, that the number of labelled cells per 1 mm length of interfollicular epidermis is a satisfactory measurement of labelling index in the hairless hamster.

However, this method may not be applicable to measuring labelling index in other species, such as the human skin, where the population density of basal and parabasal cells can vary enormously per unit length of interfollicular epidermis (e.g. normal epidermis and psoriatic epidermis). The Quantimet will certainly give the investigator a count of labelled cells in a defined length or a defined area of epidermis. A method for estimating the cell population in that defined length or defined area of epidermis must also be devised before a labelling index can be obtained.

Having set up the instrument for the detecting and counting of labelled cells, it is then possible to count them very quickly from field to field. The count per individual field is highly reproducible (Table 1) and the number of labelled cells per 1 mm length of interfollicular epidermis is determined. At least 20 1 mm lengths of interfollicular epidermis are measured per animal and the mean labelling index for that animal obtained. A group mean (and its standard error) was calculated for the number of animals in the group (Table 2).

DISCUSSION

It can be seen from Table 1 that measurements of epidermal area, sebaceous gland area, and counts are highly reproducible. Table 2 shows the relative reproducibility of the parameters for animal and human groups. All these measurements, epidermal thickness, sebaceous gland volume and labelling index can be performed for a group of six animals in approximately 90 min, illustrating the speed and versatility of the instrument.

It is difficult to compare the measurements of these parameters with those of other investigators. For instance, Ebling (1957) measured the average sebaceous gland volume in intact male haired rats. The mean sebaceous gland volume (± its standard error) for a group of 8 rats was $502 \pm 40 \times 10^3\ \mu m^3$. This compares with $290 \pm 34 \times 10^3\ \mu m^3$ in intact male hairless rats and $58 \pm 6 \times 10^3\ \mu m^3$ in intact male haired rats in this study. However, the rats used by Ebling were approximately 30 weeks old in comparison to the hairless rats which were 8–10 weeks old and the haired rats which were 6–8 weeks old. It is interesting to note that rats mature at approximately 8 weeks and that sebaceous glands may be stimulated to grow by increased androgen levels which arise at this period. There may, of course, be differences in the size of sebaceous glands due to different strains of rat but it would appear that sebaceous gland volume may be a function of age. Weirich & Longauer (1974) estimated mean sebaceous gland surface (MSGS) for auricular sebaceous glands in castrate and androgen treated rabbits. Their values can be converted to mean sebaceous gland volume (MSGV) by using the equation of Ebling (1957), and the results are:

	MSGS	MSGV
1st biopsy, juvenile, castrate rabbits	$1649\ \mu m^2$	$92{\cdot}5 \times 10^3\ \mu m^3$
2nd biopsy, 3 untreated castrates	$1562\ \mu m^2$	$85{\cdot}3 \times 10^3\ \mu m^3$
2nd biopsy, 3 castrates treated with Triolandren	$2070\ \mu m^2$	$130{\cdot}1 \times 10^3\ \mu m^3$

These values are similar to those obtained in the haired rat and the hairless hamster in this study. Once again, however, species and age difference will play a role.

Ebling (1957) quoted a value of $18{\cdot}8 \pm 1{\cdot}3\ \mu m$ for epidermal thickness of intact male haired rats (30 weeks old). This is similar to the results for the hairless rat as given in Table 2, although there may again be a strain and an age difference.

ACKNOWLEDGMENTS

The authors are grateful to Miss Elizabeth Forsyth and Miss Marylin Fleming for technical assistance.

REFERENCES

EBLING, F.J. (1957) The action of testosterone on the sebaceous glands and epidermis in castrated and hypophysectomised male rats. *Journal of Endocrinology*, **15**, 297.

FISHER, C. (1971) The new Quantimet 720. *The microscope*, **19**, 1.

SCHELLANDER, F. & MARKS, R. (1973) The epidermal response to subepidermal inflammation. *British Journal of Dermatology*, **88**, 363.

WEIRICH, E.G. & LONGAUER, J. (1974) Development of the auricular sebaceous glands in castrate and androgen treated rabbits. *Archiv für Dermatologie Forschung*, **249**, 381.

British Journal of Dermatology (1976) 95, 349.

The lines of Blaschko: a review and reconsideration

OBSERVATIONS OF THE CAUSE OF CERTAIN UNUSUAL LINEAR CONDITIONS OF THE SKIN

ROBERT JACKSON

Division of Dermatology, Ottawa Civic Hospital, Ontario, Canada

Accepted for publication 10 March 1976

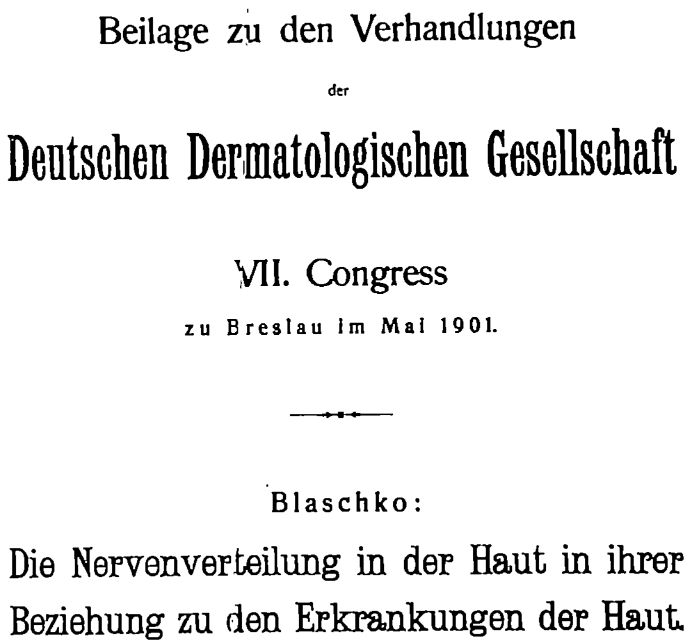
SUMMARY

Blaschko's lines are the pattern assumed by many different naevoid and acquired skin diseases on the human skin and mucosae. They were described and drawn by Blaschko 75 years ago. These lines are to be distinguished from other linear patterns such as Voight's lines, Langer's lines, and the lines of innervation of the spinal nerves. They do not follow any known nervous, vascular or lymphatic structures in the skin. The epidermis and its appendageal structures, the melanocytes, the vascular system, and the fatty hypoderm, all, separately or in combination, may be involved in the morphological manifestations which follow Blaschko's lines. Many of the naevoid skin conditions are lifelong (e.g. linear sebaceous naevus, unilateral naevoid telangiectasia); many of the acquired skin diseases (e.g. lichen striatus, linear psoriasis) are of relatively short duration (e.g. 1–2 years). The cause of the distribution pattern is unknown. It is possibly a form of human 'mosaicism' where certain specific cells or groups of cells react differently from other cells due to chromosomal abnormalities. The embryological explanation of Blaschko's lines is not at all clear. Other markers in addition to the skin findings are needed to determine the time and the nature of the change responsible for these lines. The main purpose of this article is to introduce the concept of Blaschko's lines into the medical, paramedical, and general biological fields of science. In this way, it is hoped that some inter-reaction can occur between those who regularly see Blaschko's lines and those who regularly see and study other chromosomal and embryological abnormalities.

> 'I do not know what I may appear to the world; but to myself I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me.'
>
> SIR ISAAC NEWTON, 1642–1727

In 1901 a most interesting book appeared (Blaschko). It was written and illustrated mainly by Blaschko, and was published as a supplement to the Proceedings of the German Dermatological

Address for reprints: Suite 508, 1081 Carling Avenue, Ottawa, Ontario K1Y 4G2, Canada.



Beilage zu den Verhandlungen
der
Deutschen Dermatologischen Gesellschaft

VII. Congress
zu Breslau im Mai 1901.

Blaschko:
Die Nervenverteilung in der Haut in ihrer
Beziehung zu den Erkrankungen der Haut.

FIGURE 1. Title page of Proceedings. The title can be translated as 'The nerve distribution in the skin in its relationship to the diseases of the skin'. The top portion reads 'Supplement to the proceedings of the German Dermatological Society'.

Society held in Breslau in May of 1901 (Fig. 1). It portrayed a series of lines on the human body which have been named Blaschko's lines (Fig. 2). The purpose of this paper is twofold. One is to review in some detail the contents of this book. The second purpose is to discuss and elaborate on the significance of these lines in the light of embryological knowledge and concepts developed in the last 75 years.

The book consists of about 50 large pages of text (page size 22 × 35 cm) and 26 pages of hand drawings of linear lesions on the skin of various parts of the body. Each of these pages contains from 6 to 10 separate drawings.

The text contains about 15 pages on the correlation between inflammation of the dorsal root ganglion and the involvement of the corresponding segment of skin in numerous cases of zoster. It is now accepted that the linear localization of zoster is explained by involvement of the dorsal root ganglion by the varicella-zoster virus with subsequent spread of the virus along the afferent cutaneous sensory nerves. Blaschko's studies, along with those of Head & Campbell (1900), must have been one of the earliest articles to establish an anatomical basis for the linear segmental distribution of zoster.

Next follow about 10 pages on the linear distribution of various naevoid and acquired skin diseases. There are two pages of discussion by eight members of the German Dermatological Society. Then there is a complete description of all the cases presented at the meeting.

Of the 26 pages of line drawings, the first 9 are examples of zoster. The remainder are of various linear naevoid (10 pages) and acquired (7 pages) conditions of the skin collected from the personal observations of Blaschko and from the literature available to him at that time. As one can imagine, the terms used to name these conditions are legion, e.g. naevus sebaceous lichenoides linearis, naevus verrucosus unius lateris, naevus pigmentosus unius lateris, ichthyosis linearis, vulgar eczema in lines, band-like scleroderma, segmental scleroderma, linear neurodermatitis. I have summarized and grouped all the drawings and classified the linear lesions as follows:

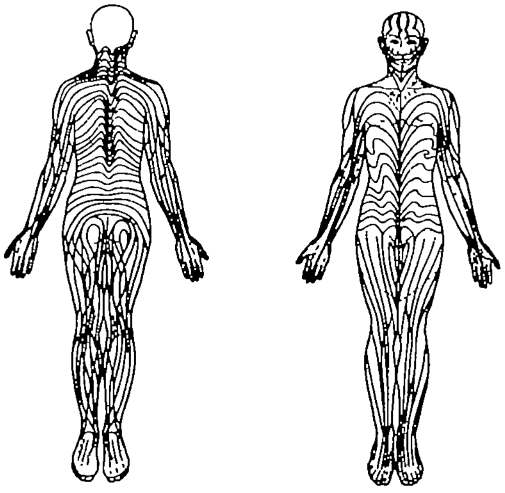


FIGURE 2. Blaschko's lines as illustrated in his 1901 article. The translation of the legend is as follows: 'A system of lines on the surface of the human body which the linear naevi and dermatoses follow. The lines have been obtained in that all cases reported in this book were outlined on a doll and then the lines, slightly schematized, were transferred to the back and front view of the human body. In the thoraco-abdominal area it was impossible to find out precisely the number of lines.'

There are 83 examples of naevoid linear skin diseases (Fig. 3). These include naevoid abnormalities of the epidermis, the sebaceous glands, the hair follicles, the cutaneous vascular system, the hypoderm, the melanocytes and sometimes combinations of these structures.

There are 63 examples of acquired linear skin disease (Fig. 4). These include examples of linear psoriasis, eczema, lichen simplex chronicus, lichen planus and scleroderma.

There are many linear naevoid and acquired lesions of the skin which follow Blaschko's lines. I have listed those that I know of in Table 1 and put in references where indicated.

For years, physicians and dermatologists have wondered why and how so many conditions of different morphology and involving different cutaneous structures could show the same basic linear pattern. A 1901 (Montgomery) article entitled 'The cause of the streaks in naevus linearis' outlines the possibilities as seen at that time. He listed the theories as follows:

'(1) The lines follow the course of the cutaneous nerves. (2) The lines run along what are called Voight's lines. (3) The lines follow the lines of cleavage of the skin. (4) The lines follow the course of the blood vessels. (5) The lines run in the metameres or segments of the body. (6) The lines lie along the embryonic sutures, and follow the trend of the tissues.'

As Montgomery (1901) pointed out, a great many observers then (as now) believed that the lines followed the course of cutaneous nerves (hence the extensive use of the word 'nerve' or 'zosteriform' in many of the synonyms). A close comparison between the lines of Blaschko and the cutaneous nerves —either in the peripheral nerve lesions or in the areas of sensory supply of the spinal cutaneous nerve (dermatomes) (Fig. 5)* shows that there is no way that Blaschko's lines can be related to the cutaneous nerves. Note particularly that in Blaschko's lines there is a V-shape over the upper spine, an S-shape on the abdomen, and an inverted U-shape from the breast area onto the upper arm

---

* It is recognized that there are differences between the locations given for the dermatomes by several authors. For a discussion of these differences, see DE JONG, RUSSELL N. (1969) *The Neurologic Examination*, 3rd ed, pp. 71–77. Hoeber Medical Division, New York. I have used the localization of KEEGAN, J.J. & GARRETT, F.D. (1948) The segmental distribution of the cutaneous nerves in the limbs of man. *Anatomical Record*, **102**, 409.

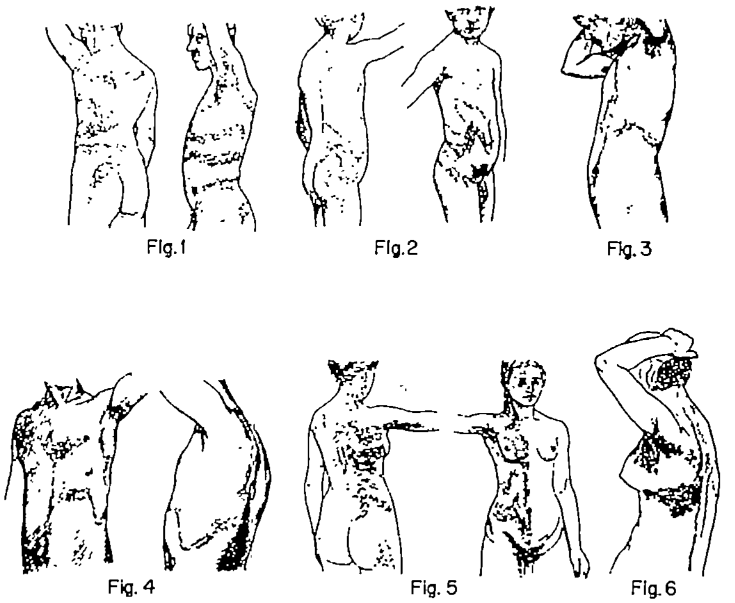


FIGURE 3. Chart XII of Blaschko's article, showing an example of his drawings of linear naevoid skin conditions. Figs 7–9 have been cropped to leave space for the legend.

Fig. 1. Naevus unius lateris, Universitätsklinik Breslau (Photogr. 1266).
Fig. 2. Naevus verrucosus linearis, nach Alexander-Blaschko (s. Lit.).
Fig. 3. Strichförmiger Naevus. Universitätsklinik Breslau (Photogr. 1603).
Fig. 4. Nervennaevus. Universitätsklinik Breslau (Photogr. 1012. 1013).
Fig. 5. Naevus unius lateris, nach Kaposi, Atlas der Hautkrankheiten.
Fig. 6. Nach Esmarch-Kulenkampf (s. Lit.).

(Figs 6 and 7). There are perpendicular lines down the front and back of the lower extremities. None of these can conceivably be related either to the cutaneous sensory nerves or to the areas of skin that they supply. On the upper extremities there does seem to be a resemblance to the anatomical location of the motor (or muscular) nerves (Fig. 9).

Voight's lines (Fig. 8) are the boundaries of the areas of distribution of the main cutaneous nerve stems and therefore constitute a totally different set of lines than those of the nerve stems themselves. But, as can be seen, many of Blaschko's lines cross Voight's lines. Also, Voight's lines do not explain the V-shape on the back or the S-shape on the abdomen.

Similarly, it is easy to dismiss Langer's lines, the course of the blood vessels or lymphatics, or a segmental origin as being reasonable explanations for the course of Blaschko's lines.

For the above reasons, Montgomery dismissed the above listed theories and postulated 'that (the) streaks may be due to the streams or trends of growth of the tissues, and to the adaptation of the embryonic sutures'.

In a portion of an article on lichen striatus entitled 'Pathogenesis of linear eruptions', Senear & Caro (1941) discussed the various theories which had been proposed to explain the configuration of these linear eruptions. The original six, as outlined by Montgomery, were included. In addition, Senear & Caro added five more possible explanations. These were:

(1) That Blaschko's lines were related to the cutaneous lymphatic vessels. (2) That the lines represented the lines of implantation of the hairs (the hair streams). (3) That the lines followed a

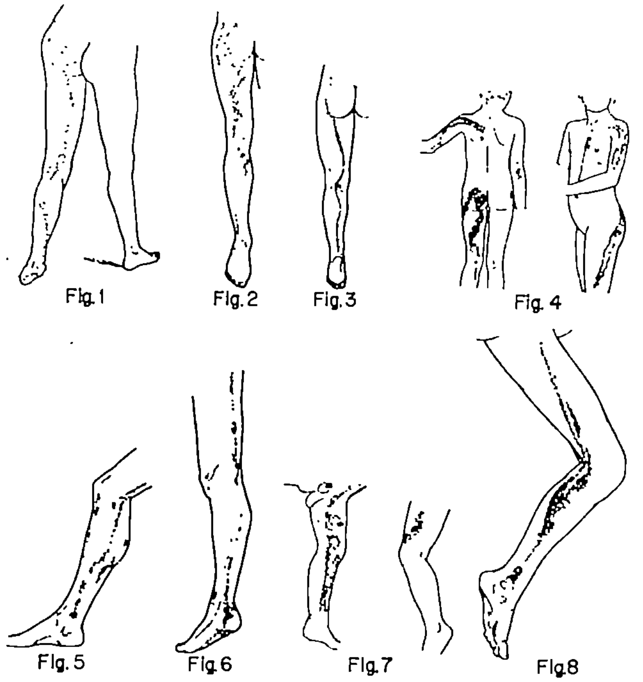


FIGURE 4. Chart XVIII of Blaschko's article showing an example of his drawings of linear acquired skin conditions. Figs 9–12 have been cropped to leave space for the legend.

Fig. 1. Ekzem im Verlaufe eines Nerven, nach Shearar (s. Lit.).
Fig. 2. Moulage de l'Hôpital St. Louis No. 2045.—Lichen plan. systématisé, localisé à la fesse et à la partie postérieure—Jambe gauche. En d'autres points, localisations typiques de lichen plan. Obs. Hallopeau. Annales 8 juin 1899.
Fig. 3. Streifenförmiges Ekzem, nach Leven (s. Lit.).
Fig. 4. Halbseitige lineäre Psoriasis, nach Blaschko (s. Anhang II).
Fig. 5. Lichen ruber in strichförmiger Anordnung, nach Ledermann (s. Anhang XIb).
Fig. 6. Neurodermitis circumscripta, etc. nach Touton (s. Lit.).
Fig. 7. Strichförmiges lichenoid-ekzematöses Exanthem am linken Arm und durch die innere Grenzlinie des vorderen Verästelungsgebietes des Beines nach hinten scharfbegrenztes gleichartiges Exanthem in diesem Gebiet, nach Touton (s. Anhang XXc).
Fig. 8. Streifenförmige Hautaffektion der linken und unteren Extremität, nach Heller (s. Lit.).

'neuritis' of the peripheral nerves. (4) That the lines were due to disturbances affecting the lines of junction of the dermatomes. (5) That the lines represented throwbacks to the markings in animals.

Senear & Caro found none of these theories acceptable, and concluded that Blaschko's lines were due to 'the presence of congenitally determined fragile zones in the skin'. They were not able to determine the factors responsible for the production of such zones of increased reactivity.

I wish now to spend some time commenting on possible causes of Blaschko's lines, with particular reference to genetic, embryological and morphological concepts which have been developed over the last 75 years.

Tissues involved

From the embryological point of view, the ectoderm, the neuro-ectoderm, and the mesoderm are the tissues of origin. One of the interesting observations is that in Blaschko's lines abnormalities of one or all these tissues may be present. I have seen linear epidermal naevi with involvement of the oral

TABLE 1. List of linear naevoid and acquired skin diseases which follow Blaschko's lines

Naevoid Skin Diseases
Linear epidermal naevus
Systematized epidermal naevus (ichthyosis hystrix)
Linear sebaceous naevus of Jadassohn
Comedone naevus (Nabai & Mehregan, 1973)
Pigmented hairy epidermal naevus of Becker (Copeman & Jones, 1965)
Unilateral nevoid telangiectasia (Selmanowitz, 1970)
Unilateral systematized achromic and hypochromic naevus (Coupe, 1967)
Zosteriform lentiginous and cellular naevus (Matsudo *et al.*, 1973)
Naevus angiolipomatosus (Howell, 1965)
Acquired Skin Diseases
Linear scleroderma (band-like) (Rubin, 1948)
Linear lichen planus
Lichen striatus
Linear eczema
Linear lichen simplex chronicus
Incontinentia pigmenti
Linear porokeratoses of Mibelli (Mikhail & Wertheimer, 1968)
Linear vitiligo*

* It should be noted that linear vitiligo has not been mapped in enough detail to be certain that it follows Blaschko's lines.

mucosa. They have also been reported as occurring on the nasal (Fig. 10) and vulvar mucosa (Brown & Gorlin, 1960). Clearly, then, more than one embryological tissue is involved.

Another factor which clearly excludes the epidermis as the sole tissue of origin of the defect or weakness which results in Blaschko's lines is the fact that the epithelium (or epidermis) in the embryo grows as a continuous sheet of cells in every way, with no definite organization or orientation. There would seem to be no way that this abnormality can be determined by (or be inherent in) the epidermis.

The role of the mesoderm (or mesenchyme) must be considered.

McLoughlin (1961) (and many others), as a result of several experiments on the epidermis transplanted to different areas comes to the following conclusions. 'Firstly, that mesenchymal factors are probably of widespread significance in influencing and maintaining the differentiation of overlying epithelia; and secondly, that since the differentiation of the early embryonic epidermis of the chick is susceptible to modification by such factors, the maintenance as well as the appearance [i.e. formation] of epidermal derivatives may depend on the underlying mesenchyme.'

A detailed review of the differentiation of the epidermis and epidermal derivatives has been given by Wessels (1967). His conclusions are that 'mesenchymal cells appear to regulate the rate and type of differentiation of both embryonic and adult surface epidermis and the growth of hair and feathers. The control of epithelial cell mitosis appears to be a common feature of this regulator, as is the apparent passive, but essential, role of the mesenchyme in acting as a substrate to which the germinal cells can attach and so remain in a proliferative condition. It is not clear what other types of information pass between mesenchyme and epithelium to serve during regulation of the epidermal cytodifferentiation process phase *per se* . . . Whether mesoderm acts by contributing the type of specific synthesis carried on by epidermis or by influencing the relative time that synthesis occurs as cells move out,

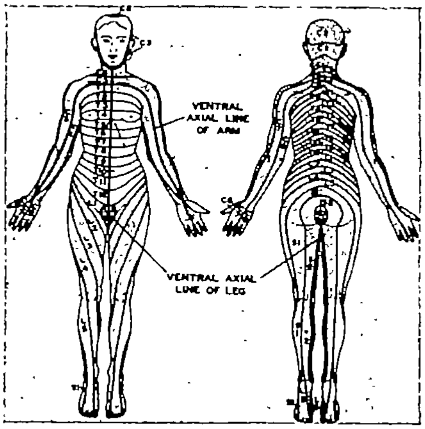


FIGURE 5. Schematic drawings of areas of innervation of the spinal cutaneous nerves (dermatomes). (Reproduced by permission from Keegan and Garrett, 1948, p. 411.)

or both, is uncertain.' He mentions that vitamin A may play a role as an extrinsic cue in this regulatory process.

Admitting the role of the mesoderm, where does this get us ? 'If the dermis controls the epidermis, what controls the dermis ?' (Whimster, 1973) The mesoderm is a complex structure embryologically, which can give rise to the following: skeletal parts and muscles, bony scale and dentine, organs of excretion and reproduction, supporting materials such as cartilage, connective tissue, ligaments, tendons and notochord of some vertebrates, circulatory system, including heart and vessels and the lining of the coelomic cavity. Also, one must include certain tissues such as nervous tissue. For example, Wessels and Roessner (1965) reported that in developing vibrissae in mouse embryos, branches of the cranial nerves extend into the dermal condensations as those aggregates of cells first become apparent. Do these thick nerve bundles act as an inductive force in the mesoderm and stimulate the

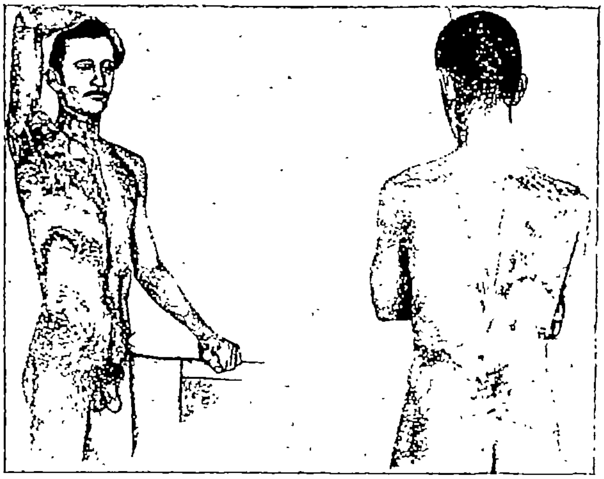


FIGURE 6. Systematized epidermal naevus. (Reproduced from HUTCHINSON, J. (1900) *Archives of Surgery*, Vol. IX, Plate CXXXII, no page. West, Newman & Co., 54 Hatton Garden, London.) Note S-shape on right loin and V-shape over vertebral area.

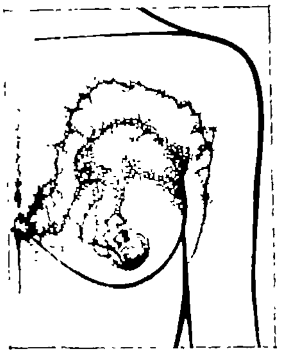


FIGURE 7. Linear epidermal naevus on left breast and left arm in a 30-year-old woman. Note inverted U-shape. The mid-line linear pattern is also shown.

formation of the dermal condensations? If so, what causes the nerves to grow to the correct points that subsequently will form the rows of vibrissae?

I think it reasonable to assume that the localization of Blaschko's lines will be found to be determined, at least in part, by the dermal tissues. But the dermis is a mixed bag of tissues, many of which have totally different embryological origins.

Possible relationship of Blaschko's lines to mosaicism

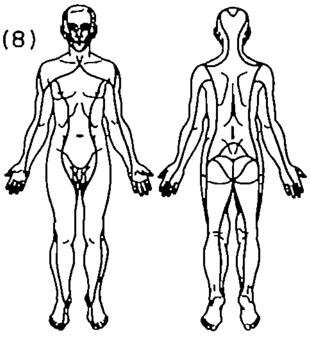
Whimster (1965) has defined a mosaic as 'a planned and organized composition built up of initially separate pieces with differing qualities, purposefully chosen and individually placed so that together they form a picture or pattern, designed to fulfil an intention'. He calls an unplanned and unorganized composite a terrazzo. It is not possible as yet to assign all biological composites to their correct group (i.e. either a mosaic or a terrazzo). I have put the word mosaic in quotation marks in the remainder of this paper to indicate that some of the described conditions may be terrazzos and not mosaics as defined above.

'Mosaicism' in the medical literature has come to mean the mixture of two (or more) genetically unlike cell lines. The difference may involve single genes, groups of genes, or entire chromosomes. Some 'mosaics' that show no obvious cellular variance may be distinguished by chromosome analysis, whereas others, easily seen from a clinical point of view, may be impossible to verify by the techniques now available.

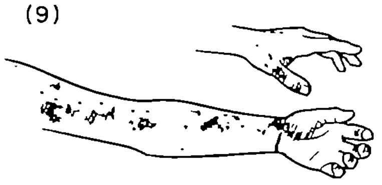
Let us look at an animal and a human 'mosaic' to see if we can get some clues as to whether or not Blaschko's lines could be a type of 'mosaicism'.

Fraser & Short (1958) reported on their studies of sheep 'mosaic' for fleece type (Fig. 11). They pointed out that while theoretically genetic 'mosaics' should be symmetrical, it is possible that initially unitary mutational events may lead to widely separated mutant areas. In sheep 'mosaicism', this is undoubtedly true. Presumably a single region of abnormal cells later forms separate regions due to cell migration. Fraser & Short explained the pattern of sheep 'mosaicism' by postulating two distinct processes during development. One is an extension in the antero-posterior direction, which is accompanied by intermigrations along dorso-ventral axes, of cells of the abnormal and normal regions. This process is deduced from the occurrence of the abnormal regions in separate patches aligned in the antero-posterior axis. The second is an extension, unaccompanied by any marked migration, which results in the separate regions being 'stretched' in the dorso-ventral direction. It should be noted that in sheep 'mosaicism' there is neither the V-pattern on the mid-dorsal (vertebral) area nor the S-shaped

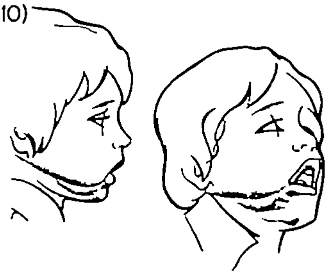
(8)



(9)



(10)



(11)

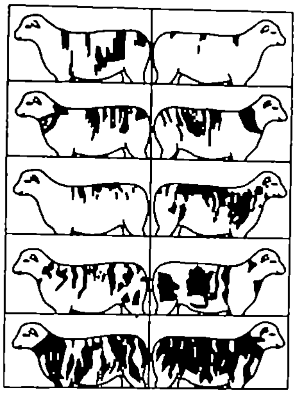


FIGURE 8. Voight's lines (after SIEMANS, H.W. (1958) *General Diagnosis and Therapy of Skin Diseases*, p. 139. University of Chicago Press, Chicago).

FIGURE 9. Unilateral naevoid telangiectasia on left arm, forearm and hand of a 16-year-old girl. The linear portion represents areas of telangiectasia. The small black dots represent brown lentigos which run along the areas of telangiectasia. Note also that the long axis of these lentigos is in the same direction. The telangiectatic portion was present at birth. The lentigos have been appearing over a period of 5 years, starting proximally and spreading distally.

FIGURE 10. Linear epidermal naevus on right side of face of a 6-year-old girl (underside of chin and postauricular area). There was also an area of linear leukokeratosis on the right buccal mucosa starting at the right oral commisure and extending posteriorly for 3 cm.

FIGURE 11. Patterns of fleece 'mosaicism'. (Reproduced with permission from Fraser and Short, 1958, p. 203.)

whorls on the flanks and lateral abdomen. It would seem unlikely that fleece 'mosaicism' in sheep could be used as an animal model or prototype of Blaschko's lines.

Davis & Shaw (1964) reported a patient with a mosaic-like arrangement consisting of bilaterally alternating squares of numerous freckles and normal skin (Fig. 12). Chromosome studies of primary fibroblast cultures showed normal human idiograms. Examination of buccal smears eliminated X-chromosome mosaicism. Haemoglobin electrophoresis showed the characteristics of sickle-cell trait. It is obvious that the pattern illustrated is not that of Blaschko's lines.

Other examples of 'mosaicism' can be found in butterflies and moths and in vertebrates. In humans, in most cases where chromosome 'mosaicism' can be shown, they have been discovered by primary or secondary sexual anomalies, or various congenital central nervous system abnormalities, or are examples of blood 'mosaicism'. None of these that I know of has ever been shown to occur with a patient who has lesions following Blaschko's lines.

Despite the fact that, in general, patients who have the various manifestations in Blaschko's lines rarely have other members of the family with similar abnormalities, it is still possible, as Holmes (1974) has indicated, that Blaschko's lines represent an inborn error of morphogenesis due to a

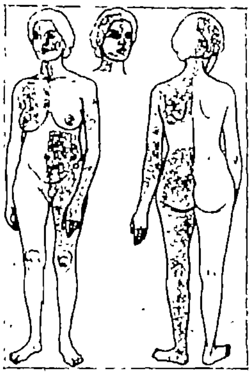


FIGURE 12. Pattern of pigmenting 'mosaic'. (Reproduced with permission from Davis and Shaw, 1964, p. 1386.)
Diagrammatic representation of involved and non-involved skin areas. The intensity of the stippling is not related to the relative concentration of lentigos.

single mutant gene. This has never been demonstrated. Adam & Richards (1973) reported in detail two unrelated families in which only one monozygotic twin demonstrated ichthyosis hystrix.

Meirowsky's 1942 *studies*

Meirowsky (1942a, b), in two long articles, illustrated and discussed several findings relative to Blaschko's lines. His collections of cases and his observations on patterned lesions in man and animals are fascinating. Unfortunately, when it comes to genetic and embryological interpretations of these observations, his use of terms does not allow one to understand clearly what he is talking about.

He pointed out first that the development of the epidermis is non-segmental and, as has been stated before in this paper, the epidermis alone can in no way explain the pattern of Blaschko's lines. He suggested that the normal stripe markings of newborn hairless dogs are basically linear pigmented naevi. This is untenable, unless one has a definition of naevi so broad as to be meaningless. He gave examples of the inheritance of linear naevi in the same location in uni-ovular twins and in parent and sibling. His list of paratypical factors not founded on genes includes the embryonal development itself, the endocrine organs, mechanical effects, differences in temperature, trauma and sunlight; he gives no explanation as to how these work. He explained the unilateral characteristic of some epidermal naevi as being due to an anomaly of one of the two cells after the first division of the fertilized egg. He mentioned the occasional localization of moles in phylogenetically important regions (such as neighbourhood of clefts, sacrolumbar regions, spine, nipples, and eyebrows). Some of his drawings are of facial epidermal naevi following Blaschko's lines. He ended by stating 'in certain cases one side of the body as well as the regions of linear naevi and diseases are "loci minoris resistentiae"'. This, of course, gets us nowhere.

General comments

One could visualize certain underlying genetic and embryological abnormalities producing the naevoid group of diseases which follow Blaschko's lines. The clinical characteristics (such as unilateral involvement, frequent presence at birth or shortly thereafter, permanence, the involvement of multiple embryological tissues, the location on mucosae, the presence in twins or in families) certainly support this concept. But it becomes more difficult to visualize a genetic or embryological background for the acquired linear skin diseases where there is no visible preceding or subsequent abnormality and the only uniting factor is the pattern assumed.

I have been unable even to make a guess at what stage of development the changes occur which could provide a mechanism by which the localization of Blaschko's lines is determined. It would be helpful to tie in Blaschko's lines with some other dateable embryological event. Unfortunately, because Blaschko's lines are so poorly known, possible tie-ins could easily have been missed.

I have no answers to explain the pattern of localization of Blaschko's lines. If nothing else, I hope this article will awaken the interest of those who commonly deal with demonstrable chromosomal, genetic, and embryological abnormalities. Meanwhile, the inadequate term 'loci minoris resistentiae' is all we can offer.

ACKNOWLEDGMENTS

Figs 7, 9 and 10 were made by Mr Michael Duguay, medical illustrator, Ottawa Civic Hospital. Dr E. Liepa and Dr W. E. Hirte did the translations from the German.

REFERENCES

Adam, J.E. & Richards, R. (1973) Ichthyosis hystrix (epidermolytic hyperkeratosis) discordant in monozygotic twins. *Archives of Dermatology*, **107**, 278.

Blaschko, A. (1901) Die Nevenverteilung in der Haut in ihrer Beziehung zu den Ekrankungen der Haut. *Beilage zu den Verhandlungen der Deutschen Dermatologischen Gesellschaft VII Congress* Breslau, May.

Brown, H.M. & Gorlin, R.J. (1960) Oral mucosal involvement in nevus unius lateris (ichthyosis hystrix). *Archives of Dermatology*, **81**, 509.

Copeman, P.W.M. & Jones, E.W. (1965) Pigmented hairy epidermal nevus (Becker). *Archives of Dermatology*, **92**, 249.

Coupe, R.L. (1967) Unilateral systematized achromic naevus. *Dermatologica*, **134**, 19.

Davis, D.G. & Shaw, M.W. (1964) An unusual human mosaic for skin pigmentation. *New England Journal of Medicine*, **270**, 1384.

Fraser, A.S. & Short, B.F. (1958) Studies of sheep mosaic for fleece type. *Australian Journal of Biological Sciences*, **2**, 200.

Head, H. & Campbell, A.W. (1900) The pathology of herpes zoster and its bearing on sensory localization. *Brain*, **XXIII**, 333.

Holmes, L.B. (1974) Inborn errors of morphogenesis: a review of localized hereditary malformations. *New England Journal of Medicine*, **291**, 763.

Howell, J.B. (1965) Nevus angiolipomatosus versus focal dermal hypoplasia. *Archives of Dermatology*, **92**, 238.

Matsudo, H. Reed, W.B., Homme, O., Bartok, W. & Horowitz, B. (1973) Zosteriform lentiginous naevus. *Archives of Dermatology*, **107**, 902.

Meirowsky, E. (1942) Moles and malformations of the skin in their relationship to inheritance and phylogenesis (new and old investigations). (a) Part I: Conditions that determine the formation of moles and malformations of the skin. *British Journal of Dermatology and Syphilology*, **54**, 99. (b) Part II: Conditions that determine the location of moles and malformations of the skin, *Ibid*, 129.

McLoughlin, C.B. (1961) The importance of mesenchymal factors in the differentiation of chick epidermis. II. Modification of epidermal differentiation by contact with different types of mesenchyme. *Journal of Embryology and Experimental Morphology*, **9**, 385.

Mikhail, G.R. & Wertheimer, F.W. (1968) Clinical variants of porokeratosis (Mibelli). *Archives of Dermatology*, **98**, 124.

Montgomery, D.W. (1901) The cause of streaks in naevus linearis. *Journal of Cutaneous and Genito-Urinary Diseases*, **XIX**, 455.

Nabai, H. & Mehregan, A.H. (1973) Nevus comedonicus. *Acta dermato-venereologica*, **53**, 71.

Rubin, L. (1948) Linear scleroderma: association with abnormalities of the spine and nervous system. *Archives of Dermatology and Syphilology*, **58**, 1.

Selmanowitz, V.J. (1970) Unilateral nevoid telangiectasia. *Annals of Internal Medicine*, **73**, 87.

Senear, F.E. & Caro, M.R. (1941) Lichen striatus. *Archives of Dermatology and Syphilology*, **43**, 116.

Wessells, N.K. (1967) Differentiation of epidermis and epidermal derivatives. *New England Journal of Medicine*, **277**, 21.

Wessells, N.K. & Roessner, K.D. (1965) Nonproliferation in dermal condensations of mouse vibrissae and pelage hairs. *Developmental Biology*, **12**, 419.

Whimster, I.W. (1973) The natural history of endogenous skin malignancy as a basis for experimental research. *Transactions St John's Hospital Dermatological Society*, **59**, 195.

Whimster, I.W. (1965) An experimental approach to the problem of spottiness. *British Journal of Dermatology*, **77**, 397.

British Journal of Dermatology (1976) **95**, 361.

The effect of collagenase on surface markers of human peripheral lymphocytes

TULIP TAN

The Department of Chemical Pathology and Dermatology,
Westminster Hospital, London SW1

Accepted for publication 26 March 1976

SUMMARY

The effect of collagenase on the 'T' and 'B' markers of peripheral blood lymphocytes was studied in five healthy male blood donors. No significant difference between untreated and collagenase treated lymphocytes was found. Collagenase is recommended for use in liberating living cells from skin biopsy specimens.

Tan, Byrom & Hayes (1975) described a method of obtaining viable infiltrating cells from skin biopsies of skin reticulosis and lichen planus using collagenase and mechanical disaggregation. T lymphocytes were differentiated by the sheep red blood cell (SRBC) technique (Jondal, Holm & Wigzell, 1972) and B lymphocytes by immunofluorescence (Dickler & Kunkel, 1972; Wilson & Nossal, 1971; Lay & Nussenzweig, 1968; Ross *et al.*, 1973). They found a predominance of T lymphocytes in mycosis fungoides, Sézary syndrome and lichen planus and only a few B lymphocytes.

While studying three further cases of lichen planus, it was noted that no immunoglobulin-bearing lymphocytes were demonstrated. The numbers binding SRBC varied between 36 and 98%. It was considered that the collagenase may have stripped immunoglobulin from the surface of B cells whilst leaving relatively unaffected the SRBC receptors.

The purpose of this communication was to find out the effect of collagenase on the surface markers of peripheral blood lymphocytes. The conditions of the experiment were made as similar as possible to that described in the separation of lymphoid cells from the skin lesions (Tan *et al.*, 1975).

METHOD

Lymphocytes from healthy blood donors were separated from 20 ml of heparinized blood on lymphoprep (Nygard) as described by Boyum (1968).

The cells were washed three times with medium TC.199, counted and viability assessed by the trypan blue exclusion technique. The cells were then divided into three aliquots. 3 and 5 mg of collagenase (Sigma, London) were added to the second and third aliquots respectively. The final volumes of the three aliquots were made up to 10 ml with medium TC.199, making a final concentration of 0·3 and 0·5 mg/ml respectively. They were kept at 4°C overnight (i.e. 17 h).

On the second day, these three aliquots of cells were incubated at 37°C for $1\frac{1}{2}$ h, then washed three times with medium TC.199 (to remove the collagenase). Viability was again assessed.

Each aliquot was then divided into two volumes. The first volume of cells was tested for T and B cells. The second volume of cells was then incubated at 37°C overnight (i.e. 20 h) in 20% autologous serum (to allow surface markers which might have been stripped by collagenase to regenerate). The next day the cells were washed three times with medium. Viability was assessed and T and B cells enumerated.

T-cell enumeration

Equal volumes of washed 1% sheep red blood cells (SRBC) (Wellcome Reagents Ltd), suspended in inactivated fetal calf serum (IFCS), and lymphocyte cell suspension were mixed. After incubation at 37°C for 15 min, the cells were centrifuged at 200 *g* at 4°C for 10 min and left at 4°C overnight before counting. The cells were then gently re-suspended by shaking and examined by phase contrast microscopy. Cells binding three or more SRBC were considered positive. At least one hundred lymphoid cells were counted.

B cell enumeration

The lymphoid cell suspension was incubated at 4°C for 20 min with polyvalent fluorescein-conjugated goat anti-human Ig serum, (anti-IgG, IgA, IgM, H and L chains, (Behringwerke, H.G.) at a dilution of 1:9). The cells were washed three times with phosphate buffered saline at 4°C and kept on ice until examined with a Leitz orthoplan microscope equipped with a mercury vapour HB200 lamp and fluorescence vertical illuminator. Cells with fluorescent spots scattered on their surfaces were considered positive. Fifty to one hundred cells were counted alternately under fluorescent and conventional light.

TABLE 1. Values of viability of lymphocytes

Donor	Day	% viable with no collagenase	% viable with 3 mg collagenase	% viable with 5 mg collagenase
1	1	90	90	90
	2	89	70	74
	3	70	68	61
2	1	89	89	89
	2	85	69	60
	3	54	59	48
3	1	80	80	80
	2	65	49	50
	3	50	47	52
4	1	85	85	85
	2	64	75	71
	3	67	69	70
5	1	88	88	88
	2	79	74	75
	3	75	72	75

RESULTS

From Table 1, it can be seen that the viability of the lymphocytes tended to decline slightly with each successive day.

Tables 2 and 3 show that the values of T and B cells are not significantly different in the untreated and treated aliquots of lymphocytes.

TABLE 2. Values for T cells

Donor	% of T cells with no collagenase		% of T cells with 0·3 mg/ml collagenase		% of T cells with 0·5 mg/ml collagenase	
	Day 2	Day 3	Day 2	Day 3	Day 2	Day 3
1	77	75	83	83	90	90
2	76	72	84	60	80	*
3	46	23	55	*	70	42
4	72	50	59	*	60	62
5	74	60	81	80	81	70
Mean	69	56	72	74	76	66

* Too few cells to count <50.

TABLE 3. Values of immunoglobulin bearing cells

Donor	% of AGM Ig cells with no collagenase		% of AGM Ig cells with 0·3 mg/ml collagenase		% of AGM Ig cells with 0·5 mg/ml collagenase	
	Day 2	Day 3	Day 2	Day 3	Day 2	Day 3
1	17	19	30	30	20	20
2	30	20	20	25	30	23
3	20	23	25	27	23	17
4	28	30	*	*	16	17
5	20	21	17	20	19	20
Mean	23	23	23	25	22	19

* Too few cells to count <50.

DISCUSSION

It was found that collagenase had no effect on the B cell markers of peripheral lymphocytes. The mean B lymphocyte count after treatment with 0·3 and 0·5 mg/ml collagenase were 23 and 22% compared to 23% in the aliquot untreated with collagenase. T lymphocyte count appeared to be higher in donors 1, 2, 3 and 5 in the aliquots treated with collagenase. However, in donor 4, the reverse was true.

The mean T cell counts in the three aliquots of 69, 72 and 76% showed that collagenase probably had no effect on T lymphocytes, the small difference being within the limits of experimental error. The difference of T cell counts on the second and third day could be due partly to the decrease in viability of the cells and partly to experimental error.

Collagenase does not seem to affect T and B lymphocyte surface markers, which is in contrast to the action of trypsin and pronase. Pellegrino, Ferrone & Theofilopoulos (1975) showed that both trypsin treated (3 ml of 0·01%) and pronase treated human peripheral lymphocytes lost their ability to rosette monkey red blood cells—described as a new method of detecting B lymphocytes: an activity which was completely recovered after 19 h culture in 10% autologous serum or fetal calf serum.

Collagenase is recommended for use in obtaining viable cells from dermal infiltrates.

ACKNOWLEDGMENTS

The author was supported by a British Council grant. Thanks are due to Professor J.R.Hobbs, Dr P.D.Samman, Dr P.W.M.Copeman and Dr D.Brigden for their invaluable help and encouragement. Thanks are also due to the donors and doctors of the Blood Transfusion Department of the Westminster Hospital for the blood samples.

REFERENCES

Boyum, A. (1968) Separation of Leucocytes from blood and bone marrow with special reference to factors which influence and modify sedimentation properties of haematopoietic cells. *Scandinavian Journal of Clinical Laboratory Investigation*, **21**, Suppl. 97, 1.

Dickler, H.B. & Kunkel, H.G. (1972) Interaction of aggregated gamma globulin with B lymphocytes. *Journal of Experimental Medicine*, **136**, 191.

Jondal, M., Holm, G. & Wigzell, H. (1972) Surface markers on human T and B lymphocytes. *Journal of Experimental Medicine*, **136**, 207.

Lay, W.H. & Nussenzweig, V. (1968) Receptors for complement on leucocytes. *Journal of Experimental Medicine*, **128**, 981.

Pellegrino, M.A., Ferrone, S. & Theofilopoulos, A.N. (1975) Rosette formation of human lymphoid cells with monkey red blood cells. *Journal of Immunology*, **115**, 1065.

Ross, G.D., Polley, M.J., Rabellino, E.M. & Grey, H.M. (1973). Two different complement receptors on human lymphocytes. *Journal of Experimental Medicine*, **138**, 798.

Tan, R.S-H., Byrom, N.A. & Hays, J.P. (1975) A method of liberating living cells from the Dermal Infiltrate—Studies on skin reticuloses and lichen planus. *British Journal of Dermatology*, **93**, 271.

Wilson, J.D. & Nossal, G.J.V. (1971) Identification of human T and B lymphocytes in normal peripheral blood and in chronic lymphocytic leukaemia. *Lancet*, **ii**, 788.

British Journal of Dermatology (1976) **95**, 365.

Infantile eczema

A LONG-TERM FOLLOW-UP STUDY

KEITH MUSGROVE AND JOHN K.MORGAN

South Teesside Department of Dermatology, Carter Bequest Hospital, Middlesbrough, Cleveland

Accepted for publication 23 January 1976

SUMMARY

A 15–17 year follow-up study was conducted on ninety-nine patients who had suffered from infantile eczema. The persistance of the eczema and the occurrence of related conditions were noted. The persistence of eczema was shown to be greater in those patients with a positive family history of eczema and in those who had developed asthma or hay-fever. An attempt was made to see if the persistence of eczema was affected by the position of the child in the family, and some factors provoking relapses were noted.

The patients were also questioned with regard to their achievements in academic examinations, and to their social, artistic and sporting activities. The results showed a success rate in examinations not significantly higher than average. It was not possible to show if there is a particular type of atopic personality. There was no constant characteristic in social or artistic patterns. The group as a whole were normal at the milestones of early development, i.e. walking, talking and reading, and also normal with regard to weight and height.

A frequent question asked of every dermatologist by the parents of a child with atopic eczema, is how long the disability will last, and what complications and progress they may expect. In spite of the high incidence of this skin disorder, the factual evidence upon which the clinician can base his reply is limited. Sedlis (1965) has remarked that the literature on the course of the disease is most unsatisfactory in revealing the frequency with which it persists into adult life. The earlier papers on the subject were mostly in German; for example, Boddin (1930) found that nineteen of thirty-three patients still had eczema 18–20 years later. Edgren (1943) in a follow-up of infantile eczema found 21% still affected by eczema, and of those reaching 25 years or over, 30% still had some form of allergic manifestation. In Great Britain, Purdy (1953) instituted a postal enquiry to 329 patients who had attended hospital in Edinburgh with infantile eczema, and though obtaining only 30% replies (from ninety-three patients) found that one-third to one-half had sequelae 'to encumber their lives up to the age of 15–20 years', although only one-fifth had Besnier's Prurigo. Finn (1955) found similar results in a smaller postal enquiry. A comprehensive study was undertaken by Vowles, Warin & Apley (1955), who visited eighty-four of 115 patients who had been admitted to hospital in Bristol 13–22 years previously with infantile eczema. Of these patients, who presumably because of their hospital admission had a relatively severe eczema, 55% still had persistence of the skin lesions up to the age of 13 years, and 50% of them had also developed asthma. Meenan (1959), using a postal questionnaire, stated that of 42 subjects with eczema who had been in-patients as children 14–27 years before, 58% had cleared by the age of 8 years, though 30·9% had not cleared by the time of his enquiry. Burrows & Penman (1960) in a similar study to Vowles *et al.*, found that of twenty-nine cases with true infantile eczema admitted to hospital in Belfast 9–12 years earlier, the high number of twenty-four (82·8%) still had eczema, and of a total of thirty-eight patients traced with either infantile eczema, contact dermatitis or seborrhoeic eczema, only 10% were free of both skin and chest complaints.

Berlinghoff (1961) conducted a postal enquiry in Jena and found 56% of his patients continued to be affected by allergic disease, and Roth & Kierland (1964) using a postal questionnaire found only 40% of mild cases and 29% of severe cases clear after 20 years. Stifler (1965) in Baltimore noted in a 21 year follow-up that eight of thirty patients were still affected by their eczema. Rajka (1975) records a continuous course for atopic eczema in 96% of fifty cases under 6 years old, but this was reduced to 48% in the 19–45 year age group, and he comments that in his series of 1200 patients only 2% were over 45 years old. The incidence of persistence thus appears from these reports to be high, but variable.

One difficulty, upon which many authors comment, is that of defining exactly the diagnostic criteria for atopic or infantile eczema, and Rajka (1961) has pointed out that comparison of one series with another is problematical because the material may not be uniform, and the time interval to follow-up may differ. There are also uncertainties associated with postal or telephonic enquiries, compared with those series in which the patients are actually examined.

In the South Teesside Department of Dermatology one clinician has kept the diagnostic index for all the years from 1958, so that the diagnostic criteria have remained reasonably constant. As the only skin clinic in the area for many years, from 1958 it is likely to have a record of most cases of infantile eczema referred to hospital, excepting perhaps a few seen in the Paediatric Department only. Some of the same medical staff who had examined the children in infancy were still available, 15 years or so later, to examine the children again. We hoped we might be in a position to learn how the eczematous infants of 1958, and subsequent years, had fared by the time they reached school-leaving age, compared with the average normal child, and to what extent the eczema had persisted. It was hoped broadly to see what influence the atopic eczema had had upon the lives of the children, upon their scholastic and sporting merits and achievements, and upon their place in society.

There has been much debate in the past on whether there exists a characteristic atopic personality (Solomon & Beerman, 1966). Stokes, Beerman & Ingraham (1940) have given a detailed summary listing many aspects of what they consider to be the atopic personality, including feelings of insecurity, inferiority, aggressiveness, tension and a higher than average I.Q. Whether infantile eczema patients are, in fact, more intelligent than the average child is open to discussion. Piness, Miller & Sullivan (1937) and Chobot, Spadevecchia & De Sanctis (1959) found the intelligence ratings of allergic children to be normal, whereas Bayleat (1929) and Stokes *et al.* (1940) suggested a higher than average intelligence. Unfortunately, it was not felt possible in this survey to conduct I.Q. tests on our patients because of the already lengthy questionnaire. However, most of them had reached the age at which examinations, such as General Certificate of Education 'Ordinary' levels are taken, so that it seemed a survey of their success or otherwise in these examinations, as compared with the rest of the population, might be informative.

METHOD

An attempt was made to interview all patients with atopic eczema, who had first attended the South Teesside Department of Dermatology during the years 1958–1960, and who had been aged less than

5 years when originally seen. The great majority of these patients had the usual atopic flexural pattern of eczema, but also included were children with an eczema affecting at first the face and scalp (though the eruption may later have become more widespread), and a few with a petaloid pattern of eczema affecting mainly the trunk. Children with infantile seborrhoeic dermatitis were not included. There was a total of 185 such patients, nearly all of whom had been out-patients only, and 146 were traced. Thirty-nine patients could not be traced at all and were presumed to have left the area. Of the 146 patients traced, three had moved abroad, five refused to take part, one had died, and thirty-six did not respond to repeated requests asking them to take part in the survey. There remained ninety-nine patients who co-operated in the survey, which was less than we would have wished, but the 15–17 year interval inevitably produced difficulties. It may well be that some of those who did not respond were no longer affected by eczema (as there were certainly several among those who did co-operate who now had no skin trouble and who had no personal recollection of ever having had any). Some young people, innocent in this way, may have thought it all a mistake, or at any event too tedious and time-wasting a subject to evince a response.

The majority of patients interviewed came back to the out-patients department, but when this was not possible, they were visited at home. Since the patients were mostly in their mid-teens a parent was asked to be present on each occasion to help in completing a questionnaire. A physical examination was conducted to determine the present extent of the eczema.

The questionnaire drawn up involved questions on the progress of the eczema and related conditions, on family history, and on provoking factors, particularly with regard to season and clothing, and enquiries were also made concerning the education and social and physical development of the patient.

The ninety-nine patients were questioned about their educational qualifications and interests, and the parents were asked to make some assessment of their children's personalities. Each patient had his or her weight and height measured to determine if these were within the normal percentiles.

With regard to the personality of each patient; again it was not possible to undertake a detailed psychological examination, but inquiry was made of the parents' views on the personality of their child, wherever possible compared to those of siblings, in the hope that any tendency in the group as a whole might become apparent.

RESULTS

The total number of patients interviewed and examined was ninety-nine. All were Caucasians; 55 were male and 44 were female. The mean age at the time of interview was 16·17 years. The mean age of onset of the eczema was 7 months and the median age 4 months. 72 (72·16%) of the patients had developed eczema before the age of 6 months. Forty-seven patients still had eczema, as confirmed by examination, and a further ten stated they continued to be affected at times by eczema, though they were clear when interviewed. A total of fifty-seven patients (57·58%) thus still suffered from eczema up to the age of interview. A rough classification was made of those children severely, or fairly severely, affected when first seen, and those only moderately or mildly affected. The severe and fairly severe cases showed a persistence of their eczema in approximately twice as many instances as the moderate and mild cases. Though we shared with others the impression that patients with atopic eczema which was of late onset had a worse outlook than average, this was not supported by our findings; there were fifteen patients in the survey whose eczema was recorded as starting at the age of one year or later, and the prognosis for these was the same as for the group as a whole.

Related conditions

Of the ninety-nine patients, twenty-eight (28·28%) had also developed asthma, the mean age of onset being 4·6 years (median age 3 years). Of this group twenty-three patients (82·24%) had developed asthma by the age of 7 years. Twenty-two (78·57%) of these patients were male, and six (21·43%) were female. Twenty (71·42%) were still affected, and eight (28·57%) (all male) had cleared up—(the mean age of clearing was 12·75 years). Of the twenty-eight patients who had had asthma, twenty-one (75·00%) were still affected by their eczema at the time of interview. Thirty-two patients developed hay-fever (20 males, 12 females), the mean age of onset being 9 years (median age 10 years). None of these had cleared up by the time of interview, and of them twenty-two (68·75%) still had eczema.

The incidence of hay fever did not bear any relation to the severity of the original eczema, cases occurring with equal frequency whether the skin disease was regarded as severe, fairly severe, moderate or mild. By contrast there did appear a strong correlation between the later development of asthma and the degree of severity of the eczema; of the twenty-eight asthma patients, twenty-three had eczema which we originally classified as severe or fairly severe, whereas in only five instances had asthma developed in patients whose eczema we labelled moderate or mild.

Enquiry was made into the situation when a child had both eczema and asthma, whether the two conditions tended to occur together, or alternately. There were twenty-eight patients who had had both eczema and asthma, but in ten there was no constant relationship between the severity of the eczema and that of the asthma. Of the remainder, six said there was a direct relationship, i.e. the two conditions were inclined to occur or be worse together. A further four found an inverse relationship between the two, i.e. the conditions occurred or were severe alternately. The remaining eight patients did not suffer from their eczema and asthma at the same time of life.

Family history

The family history of eczema and related conditions was assessed in two ways: (1) Close family history, i.e. parents and siblings (information obtained from ninety-eight patients). (2) General family history, i.e. any known relations (information obtained from ninety-seven patients).

(1) *Close family history*

Sixteen (16·32%) of the patients had a parent who had suffered from infantile eczema. However, another twenty stated that their parents had had some other form of skin complaint such as suspender dermatitis, dry irritable skins, etc. The results obtained for eczema, asthma and hay-fever are summarized in Table 1. Twenty patients had no close family history of eczema, asthma or hay-fever.

TABLE 1. Number of patients with a close family history suffering from eczema, asthma or hay-fever

No. of patients who had	Eczema	Asthma	Hay-fever
A parent affected	16 (16·32)* (+20?)	12 (12·24)	11 (11·22)
At least one sibling affected	31 (31·62)	19 (19·36)	16 (16·32)
2 or more siblings affected	12 (12·24)	1 (1·02)	3 (3·06)

* Percentage in parentheses.

(2) *General family history*

Fifty-eight (59·79%) of the eczema patients were found to have a relative (or relatives) with eczema, fifty-three (54·64%) with asthma, forty-one (42·27%) with hay-fever, seventeen (17·53%) with psoriasis, thirty-three (34·02%) with migraine, and twenty-seven (27·84%) with urticaria.

These results were also used to determine whether there is a tendency for each aspect of the eczema/asthma/hay-fever complex to run 'true to type':

(1) *Close family history*

Of 28 patients with asthma —11 had a close relative affected, i.e. 39·29%
Of 70 patients without asthma —14 had a close relative affected, i.e. 20·00%
Of 31 patients with hay-fever —16 had a close relative affected, i.e. 51·61%
Of 67 patients without hay-fever—10 had a close relative affected, i.e. 14·95%

(2) *General family history*

Of 28 patients with asthma —21 had a relative affected, i.e. 75·00%
Of 69 patients without asthma —32 had a relative affected, i.e. 46·38%
Of 31 patients with hay-fever —18 had a relative affected, i.e. 58·06%
Of 66 patients without hay-fever—23 had a relative affected, i.e. 34·85%

These results suggest a strong tendency for the asthma and hay-fever aspects of the eczema/asthma/hay-fever complex to run 'true to type'.

In order to test whether the eczema was more likely to persist in an only child and whether the child's position in the family affects the persistence of the eczema, the persistence of the eczema was compared for four groups of children, i.e.: (1) Only children; (2) 1st born children in a family with more than 1 child; (3) 2nd born children; (4) 3rd born children and all others. The results are given in Table 2. These suggest a slight downward trend for persistence the later a child comes in a family.

These results are not the same as those of Vowles *et al.* (1955) who showed a tendency for the duration to be less with an only child and greater when there were many children in the family.

TABLE 2. The persistence of eczema related to the position of a child in the family

		Still affected by eczema	
	Total	Numbers	(%)
(1) Only children	9	6	66·67
(2) 1st born	24	14	58·33
(3) 2nd born	35	20	57·14
(4) 3rd born+	28	14	50·00

Provoking factors

Season. There was no constant seasonal influence on the eczema. Of the total of ninety-nine patients, thirty-six stated their eczema was affected by the season. Of these, nineteen were worse in Winter, twelve in Summer, one in Spring, one in Autumn and Spring, one in mid-Summer and mid-Winter, and two stated they were better in Summer. The remainder noticed no influence of the seasons, which is in contrast to the observations in the U.S.A. of Osborne & Murray (1953).

Clothing. Forty patients stated their eczema was exacerbated by clothing. Of these, twenty-three were made worse by wool, twelve by nylon, two by both wool and nylon, two by rough clothing, and one by fluffy blankets. These suggestions were not checked by patch testing, etc. and can only be quoted as patients' suggestions.

Other factors. Forty-nine said they were provoked by other factors—the most important being heat (nineteen cases) and excess immersion in water (six cases).

Weight & Height. The weight and height measurements for both boys and girls were set out on standard charts prepared by J.M.Tanner & R.H.Whitehouse (1966) according to age. Both boys and girls conformed closely to the standards for weight and for height, and in this series the atopic individuals were ***not*** below the normal percentiles for these measurements, though one's clinical impression had been that they often were.

Milestones. The age at which each child began to walk, to talk and to read was obtained from the parent in ninety-two of the eczema cases. Occasionally the parent was not certain of the exact age and could only state whether the child was normal, early or late in development for any particular characteristic. It can be seen that in this group the occurrence of infantile eczema did not appear to affect the early development of the children.

Education. Educational qualifications were assessed by the number of General Certificate of Education 'Ordinary' or 'Advanced' level, or Certificate of Secondary Education examinations which had been passed, and also the number which it was the intention to take in the future. For various ethical considerations, which we accepted, the Local Education Authorities were reluctant to furnish us with individual examination results from unaffected normal subjects, matched for age and sex with our patients. They were therefore compared to figures taken from the Statistics of Education (H.M.S.O.) which gives a full list of all examinations taken and passed in England and Wales in any year. Since most of our patients were still at school (mean age being 16·17 years), it was not possible to compare all their final results with those for the country as a whole in any particular year. However, there were a number of points on which comparison was possible.

The examination results for the country as a whole, until very recently, appear to have varied little from year to year. It was therefore considered justifiable for the examination results of the infantile eczema patients to be compared to those taken from the Statistics of Education 1972, Volume 2, 'School-leavers during the Academic Year 1971–72' (H.M.S.O.).

Comparing those of our patients who had attempted, or who intended to attempt, 'O' levels and 'A' levels with the numbers who attempted these in 1972 there were: Fifty-one of ninety-nine (51·05%) attempt 'O' levels compared with 42% from the Statistics of Education. Twenty-five of ninety-nine (25·25%) attempt 'A' levels compared with 18% from the Statistics of Education. Of those who had already taken examinations (66 patients), twenty-two (33%) had no passes of any kind compared with 43% from the Statistics of Education.

Only three of the patients had actually taken G.C.E. 'A' levels, but another twenty-two said they would be attempting them in the future. If one makes the presumption that approximately the same percentage of these will pass as in 1972 in the country as a whole, a final figure of 22% would be obtained for patients who achieve one or more 'A' level.

These results are summarized in Table 3.

Personality. A parent of each patient was asked to make an assessment of the patient's personality compared with that of any siblings, where possible. Reasonably clear answers were obtained for seventy of the ninety-nine patients. Of these seventy, thirty-eight were still affected by eczema.

TABLE 3. Examination attempts and results in infantile eczema patients, and in the country as a whole

	Statistics of education (%)	Infantile eczema patients (%)
Attempt 'O' levels	42	52
Attempt 'A' levels	18	25
No passes of any kind	43	33
At least 3 'O' levels	23	29
At least 5 'O' levels	21	21
1 or more 'A' levels	16	22(?)

The individuals of the group were given many different characteristics and there did not appear to be any obvious overall tendency to a particular type of personality.

DISCUSSION

The results of this survey with regard to age of onset of the eczema and other conditions confirm generally accepted views (Champion & Parish, 1972). The degree of persistence of the eczema is comparable to that found by Vowles *et al.* (1955) and Berlinghoff (1961). Asthma was found to occur somewhat less frequently than in some previous surveys. Of interest concerning the incidence of asthma was the different distribution in the sexes, i.e. 78·57% male:21·43% female (this ratio is reduced somewhat if one takes into account the total ratio of males to females in our series of 5:4, but nevertheless the proportion of males to females is very high). Also, all the eight patients who had had asthma but were now clear of it, were male. This concurs with the findings of Brereton *et al.* (1959) and Eriksson-Lihr (1955).

The long-term prognosis of the eczema for patients who also had asthma or hay-fever was found to be worse than for the group as a whole. Of the asthma group 75·00% were still affected by eczema; of the hay-fever group 68·75% were still affected; while for the group as a whole 57·58% were still affected.

Further, the eczema was likely to persist longer in patients with a family history of eczema. Of fifty-eight patients with a positive family history (general), forty (68·97%) had a persisting eczema, but of the remaining thirty-nine with no family history, only sixteen (41·03%) were still affected.

There was a very low mortality rate (the patient who died was killed in an accident) in contrast to Vowles' and Purdy's figures who found a surprisingly high one, but this may be due to the fact that both these earlier surveys were carried out on patients who first developed their eczema, as Vowles *et al.* stated, 'before sulphonamides and antibiotics were available'.

So far as personality is concerned, it is interesting to note that of twelve patients described as 'quick-tempered', eleven were still affected by eczema. Of eight described as 'placid' none was still affected by eczema, and of eight described as 'sociable', seven were not affected by eczema. These results might be said to go some very small way towards agreeing with the detailed atopic personality suggested by Stokes *et al.* (1940) though they are obviously not conclusive, and as suggested by Purdy (1953) in his postal follow-up survey a thorough psychological study of each patient is really needed for assessment.

It can be seen from the examination studies that the educational achievements of the infantile eczema patients appeared to be only very slightly better than those for the country as a whole. The figures for patients attempting 'O' levels and 'A' levels take into consideration those who intended to attempt them in the future and so will probably be higher than the actual final figures. Bearing this in mind, no great difference for success in examinations is shown between our patients and the country as a whole.

The results for social, artistic and sporting activities did not show any striking characteristic for the group, though in the field of sport it was interesting to note that the asthma sufferers were successful in the same number as those without asthma. In fact, those asthma sufferers who were successful in sport often appeared to be rather more gifted in this field than similar non-asthma patients (holding higher team places, more often Captains of teams, etc.). This may be due to a more determined effort from these patients to overcome their disabilities.

ACKNOWLEDGMENTS

This study was undertaken with the aid of a Research Grant from the Newcastle Regional Hospital Board. We are grateful to Dr G.A. Durham who undertook some of the earlier interviews and to all the patients and their relatives who took part.

REFERENCES

BAYLEAT, R.M. (1929) The general health and mental activity of allergic children. *American Journal of the Dystrophic Child*, **37**, 1193.

BERLINGHOFF, W. (1961) Die Prognose des Säuglingsekzems. *Deutsche Gesundheitswesen*, **16**, 110.

BODDIN, M. (1930) Beitrag zur Klinik des neurogenen Ekzems in Kindesalter. *Medizinischer Klinik*, **26**, 270.

BRERETON, E.M., CARPENTER, R.G., ROOK, A.J. & TYSER, P.A. (1959) The prevalence and prognosis of eczema and asthma in Cambridgeshire school children. *Medical Officer*, **102**, 317.

BURROWS, D. & PENMAN, R.W.B. (1960) Prognosis of the eczema–asthma syndrome. *British Medical Journal*, **ii**, 825.

CHAMPION, R.H. & PARISH, W.E. (1972) Atopic dermatitis. In: *Textbook of Dermatology* (Ed. by A.J. Rook, D.S. Wilkinson and F.J.G. Ebling) 2nd edn, vol. 1, p. 295. Blackwell Scientific Publications, Oxford.

CHOBOT, R., SPADAVECCHIA, R. & DE SANCTIS, ROSE M. (1959) Intelligence rating and emotional pattern of allergic children. *American Journal of the Dystrophic Child*, **57**, 831.

EDGREN, G. (1943) Prognose und Erblichkeitsmomente bei Ekzema Infantum. *Acta Paediatrica*, **30**, Suppl. II.

ERIKSSON-LIHR, Z. (1955) Special features in allergy in children. *Acta Allergologica*, **8**, 289.

FINN, O.A. (1955) Long-term prognosis in infantile eczema. *British Medical Journal*, **i**, 772.

MEENAN, F.O.C. (1959) Prognosis in infantile eczema. *Irish Journal of Medical Science*, **398**, 79.

OSBORN, E.D. & MURRAY, P.F. (1953) Atopic dermatitis. A study of its natural course, and of wool as a dominant allergenic factor. *Archives of Dermatology and Syphilology*, **68**, 619.

PINESS, G., MILLER, H. & SULLIVAN, E.B. (1937) The intelligence rating of the allergic child. *Journal of Allergy*, **8**, 168.

PURDY, M.J. (1953) The long-term prognosis in infantile eczema. *British Medical Journal*, **i**, 1366.

RAJKA, G. (1961) Prurigo besnier (atopic dermatitis) with special reference to the role of allergic factors. *Acta dermato-venereologica*, **41**, 363.

RAJKA, G. (1975) *Atopic Dermatitis*, p. 6. W.B. Saunders, London.

ROTH, H.L. & KIERLAND, R.R. (1964) The natural history of atopic dermatitis. *Archives of Dermatology*, **89**, 209.

SEDLIS, E. (1965) Conference on infantile eczema. *Journal of Pediatrics*, **66**, 158.

SOLOMON, L.M. & BEERMAN, H. (1966) Atopic dermatitis. *American Journal of Medical Science*, **252**, 478.

STIFLER, W.C. (1965) Conference on infantile eczema—a 21 year follow-up of infantile eczema. *Journal of Pediatrics*, **66**, 166.

STOKES, J.H., BEERMAN, H. & INGRAHAM, N.R. JR. (1940) The psychoneurogenous component of cutaneous reaction mechanisms. *American Journal of Medical Science*, **200**, 560.

TANNER, J.M. & WHITEHOUSE, R.H. (1966) Growth and development records—the source data for which are taken from TANNER, J.M., WHITEHOUSE, R.H. & TAKAISHI, M. (1966) *Archives of Diseases in Childhood*, **41**, 454.

VOWLES, MARY, WARIN, R.P. & APLEY, J. (1955) Infantile eczema: observations on natural history and prognosis. *British Journal of Dermatology*, **67**, 53.

British Journal of Dermatology (1976) 95, 373.

Psychological profile of patients with atopic dermatitis

HANA F.AL-AHMAR AND AMAL K.KURBAN

Division of Dermatology, the American University of Beirut Hospital, Beirut, Lebanon

Accepted for publication 30 January 1976

SUMMARY

Tests designed to measure psychopathological characteristics common to the neurotic population were administered to patients with atopic dermatitis, patients with other dermatological disorders and to a control group of normal individuals. The parameters tested were manifest anxiety, neurosis, extroversion, depression, hypochondriasis and hysteria. The scores were statistically analyzed. The results showed that patients with atopic dermatitis responded significantly differently from patients with other dermatological diseases and from the control group in specific psychometric scales. Moreover, patients with skin conditions other than atopic dermatitis also responded significantly differently from the control group. The study clearly shows that patients with atopic dermatitis have a characteristic psychological profile not shared by the other two groups. The atopic dermatitis patients tend to be in a state of high manifest anxiety, depressed, neurotic and hypochondriac.

It is generally recognized that in skin disease more than one factor may be involved in the evolution of the disorder. Emotional factors, manifested in overt neurotic behaviour, can influence or complicate skin diseases (Obermayer, 1955). The skin serves the unique function of separating the individual from his environment and quite often is a potential source of anxiety in itself (Eller, 1974). It is not strange then, that in certain disease entities, the emotional and personality disturbances have a great impact (Obermayer, 1955; Brown, 1967). One such psychosomatic condition is atopic dermatitis.

Sheldon (1942), one of the first to describe the 'Atopic' personality, characterized such individuals as tense, nervous, depressed, introverted and apprehensive. Allerhand, Gough & Grais (1950) used a statistical technique to establish several 'atopic trait clusters'. These clusters seemed to distinguish patients with atopic dermatitis from those with cutaneous disorders of a demonstrable origin and from general medical patients. The clusters described the general factors of nervous tension, depression and fluctuating moods. Obermayer *et al.* (1952) have found that atopic patients exhibit many of the psychoneurotic traits that classify them as a subgroup within the neurotic population. Moreover, atopic patients were more homogeneous in their traits than other neurotic patients. It should be mentioned, however, that personality patterns of patients with atopic dermatitis were considered as not specific for the disease (McLaughlin, Shoemaker & Guy, 1953).

The purpose of this study was to investigate the existence of the atopic personality, i.e. to identify the consistency of occurrence of specific psychopathological behaviours in atopic patients. Any significant clustering of such behaviours would imply the existence of an atopic psychological profile, provided that this profile was not shared by either the dermatological patients with diseases other than atopic dermatitis, or the normal controls. Accordingly, atopic patients would be expected to exhibit psychological traits and behaviours that were significantly distinct from those exhibited by either the normal population or other dermatological patients.

METHOD

Subjects. Three groups of twelve subjects each, participated in the present study. The groups were categorized as follows: patients suffering from atopic dermatitis (AG), patients suffering from dermatological diseases other than atopic dermatitis (DG), and control group of normal subjects (NG). An individual was included in the AG group on the basis of history and distribution of the dermatitis. The diagnosis of atopic dermatitis (and other dermatological diseases) was made by a dermatologist (A.K.K.). In the three groups the ages of subjects ranged between 16 and 36 years. The mean ages for the AG, DG, and NG groups were 23 (s.d. = 6·5), 22 (s.d. = 5·3) and 23 (s.d. = 6·4) respectively.

Controls. Control subjects were matched with the experimental group for age, sex, education and religion. They were selected in a systematic way (to satisfy the matching criteria) from a large pool of DG and NG subjects. The DG was chosen from the pool of patients coming daily for treatment at the American University Hospital (out-patient department). The NG was chosen from the Hospital personnel (laboratory technicians, research assistants, secretaries, etc.). They were assumed to give a fairly good representation of the normal population.

Materials and procedure. Psychological data were obtained by administration of the Eysenck Personality Investory (E.P.I.) (Eysenck & Eysenck, 1964), the Manifest Anxiety Scale (M.A.S.) (Taylor, 1953) and the neurotic triad (Depression scale, Hysteria scale, Hypochondriasis scale) from the Minnesota Multiphasic Personality Inventory (M.M.P.I.) (Hathaway & McKinley, 1951).

These tests are considered as standard measures of the psychopathological characteristics common to the neurotic population, such as Manifest Anxiety (M.A.S.), Neurosis (N), Extroversion (E), Depression (D), Hypochondriasis (Hs) and Hysteria (Hy). The combination of these scales provided an item pool of 263 statements. Some of these had to be eliminated to avoid sentence repetition since some of the scales (MMPI scales and MAS) had some items in common. The final scoring, however, treated the scales as complete and separate entities. Therefore, if for example a statement was common to a few scales, the score obtained on that statement was added to the score of each scale. The final number of items given to the subjects amounted to 217 statements.

Items were randomized and then translated into Arabic by three individuals fluent in both Arabic and English. These translations were done individually and at different times. The translations that were common and/or similar among the three versions were then tape-recorded and were administered to a group of eight judges to assess the comprehensibility of the questionnaire items. Those translations that were agreed upon by six out of the eight judges were included. Necessary changes were made on the others to attain better comprehensibility.

The questionnaire was then given to the subjects. Individual testing sessions were run by a psychologist (H.F.A.) who recorded their responses. The instructions were read to the subject by the interviewer. These emphasized that the questionnaire pertained to attitudes and behaviours usually encountered in day to day activities and the subjects were asked to answer truthfully in a forced-choice manner (yes–no response).

RESULTS

The data for each subject's response were scored by the following procedure: a 'plus' was any response that agreed with the established neurotic profile of a scale. A scale score was the sum total of 'pluses' obtained within that scale. Each subject, then, had six scores, each of which represented a different type of observation. The scores were a MAS score, an E score, an N score, a D score, an Hs score and an Hy score.

The means and standard deviations of subjects' scores were computed on the six scales for the three groups and appear in Table 1.

TABLE 1. The means and standard deviations of each of the three groups for each of the six psychometric scales

Psychometric scales		Groups		
		Atopic (AG)	Derm. (DG)	Normal (NG)
Manifest anxiety	$\bar{x}$	28·17	22·00	15·17
(MAS)	s.d.	7·98	8·04	2·72
Neurosis	$\bar{x}$	17·33	14·08	9·08
(N)	s.d.	4·14	3·40	2·20
Extroversion	$\bar{x}$	11·08	11·17	10·50
(E)	s.d.	2·43	1·40	2·94
Depression	$\bar{x}$	28·00	23·17	16·83
(D)	s.d.	5·08	3·59	3·64
Hypochondriasis	$\bar{x}$	20·17	13·83	11·25
(Hs)	s.d.	10·05	7·94	2·38
Hysteria	$\bar{x}$	24·33	20·08	18·75
(Hy)	s.d.	7·24	7·12	2·26

Six 1 × 3 analysis of variance were carried out on the data. Each of these compared the three groups on a different psychometric scale. The results yielded significant differences among the three groups for each of the MAS, N and D scales, F (2, 33) = 11·20, 26·10 and 21·74, respectively, $P<0{\cdot}001$, and on the Hs scale, F (2, 33) = 4·46, $P<0{\cdot}05$. Moreover, a set of orthogonal weighting coefficients was generated which allowed three comparisons to be carried out on the significant values.

Comparison 1. Response of AG versus response of DG on each of the psychometric scales.

Comparison 2. Response of AG versus response of NG on each of the psychometric scales.

Comparison 3. Response of DG versus response of NG on each of the psychometric scales.

The results of these comparisons for the three groups are summarized in Table 2.

Comparisons 1 and 2 compared AG with each of DG and NG, consecutively to see if the type of group (i.e. disease or no disease) influenced performance. Results showed that AG obtained significantly higher scores than either DG or NG on MAS, N, D and Hs. Comparison 1 showed that AG performed significantly differently from DG, with scores significantly higher on MAS, and Hs, F (1, 33) = 5·04 and 4·25, respectively, $P<0{\cdot}05$, and on N and D, F (1, 33) = 7·97 and 8·10, respectively, $P<0{\cdot}01$.

Comparison 2 revealed even more significant and intense differences in performance between AG and NG, with AG obtaining significantly higher scores on each of MAS, N and D, F (1, 33) = 22·38, 51·37 and 43·21, respectively, $P<0{\cdot}001$, and on Hs, F (1, 33) = 8·43, $P<0{\cdot}01$.

TABLE 2. *Results of comparisons carried out on significant F values for the three groups

		Comparison 1 Atopic vs Derm.	Comparison 2 Atopic vs Norm.	Comparison 3 Derm. vs Norm.
MAS	F	5·04	22·38	6·18
	P	<0·05	<0·001	<0·025
N	F	7·97	51·37	18·86
	P	<0·01	<0·001	<0·001
E	n.s.†			
D	F	8·10	43·21	13·90
	P	<0·01	<0·001	<0·001
Hs	F	4·25	8·43	<1
	P	<0·05	<0·01	>0·05
Hy	n.s.			

* Results appear in terms of F values and their probability of occurrence.

† n.s. = F not significant (from analysis of variance results).

These characteristics appear to be more intense and more apparent in AG than either of DG and NG.

Comparison 3 tested for any difference in performance between DG and NG on each of the six psychometric scales. Results were as follows: DG differed significantly from NG, with DG obtaining significantly higher scores on MAS, $F(1, 33) = 6·18$, $P<0·025$, N and D, $F(1, 33) = 18·86$ and 13·90 respectively, $P<0·001$. However, there was no significant difference between the two groups on Hs.

DISCUSSION

This study, which admittedly is a pilot one because of the sample size, utilized scientific psychological testing and statistical analysis of the scores. The results indicated that AG have characteristics that are different from DG and NG. The differences between the groups is in the intensity of specific traits (AG vs DG) and/or in the traits themselves (AG vs DG vs NG).

AG and DG were different from NG by showing some degree of psychological abnormality as defined by the scales under study. AG differed from DG in the type and degree of psychological abnormality which was more pronounced in AG. Thus AG seem to have specific characteristics that define their psychological profile in a clear cut manner and this profile is not shared with other dermatological disorders or by normal subjects. This is borne out by the fact that AG respond in a consistent manner to specific scales and not to others.

The characterization of the psychological profile of AG may be enhanced by adding other psychometric scales and expanding the sample size. Patients with atopic dermatitis are characterized by a state of high manifest anxiety, depression, neurosis and hypochondriasis. Such a characterization points to the homogeneity and uniformity of the personality of atopic dermatitis patients. It seems that such patients belong to a different and specific population. One can postulate that the psychological abnormalities in patients with atopic dermatitis may be part of their inherited diathesis or a product of the interaction of inherited potentialities with life experiences.

Obermayer (1955) claims that patients with atopic dermatitis have an abnormal personality.

This study supports this claim and specifies the nature of the abnormalities: the patient with atopic dermatitis is an introvert, depressed, highly anxious individual. The absence of significant differences in the extroversion and hysteria scales may be due to cultural influences. Such influences may inhibit or enhance specific traits in the profile. Notwithstanding this, it is apparent that there is universality and uniformity of the atopic profile across cultures.

ACKNOWLEDGMENTS

The authors wish to thank Dr U.Yaktin and Professor L.Melikian of the Psychology Department, American University of Beirut, for their advice and help. The authors wish also to thank E.R.Squibb for their financial assistance that made this study possible.

REFERENCES

ALLERHAND, M.E., GOUGH, H.G. & GRAIS, M.L. (1950) Personality factors in neurodermatitis. A preliminary study. *Psychosomatic Medicine*, **12**, 386.

BROWN, D.G. (1967) Emotional disturbances in eczema. A study of symptom-reporting behaviour. *Journal of Psychosomatic Research*, **11**, 27.

ELLER, J.J. (1974) Skin disorders and the psyche. *Cutis*, **13**, 395.

EYSENCK, H.J. & EYSENCK, S.B.G. (1964) *Manual of the Eysenck Personality Inventory*. University of London Press, London.

HATHAWAY, S.R. & MCKINLEY, J.C. (1951) *Minnesota Multiphasic Personality Inventory Manual*. The Psychological Corporation, New York.

MCLAUGHLIN, J.T., SHOEMAKER, R.J. & GUY, W.B. (1953) Personality factors in adult atopic eczema. *Archives of Dermatology and Syphilology*, **68**, 506.

OBERMAYER, M.E., BLAIR, R., FISKE, C., LEVITT, N., RUSH, S., STORKAN, M.A. & GLENN, E. (1952) Correlation of emotional status and reactivity to cutaneous stimuli in functional dermatoses. *Archives of Dermatology and Syphilology*, **65**, 291.

OBERMAYER, M.E. (1955) *Psychosomatic Medicine*. Charles C. Thomas, Springfield, Illinois.

SHELDON, W.H. (1942) *The Varieties Of Human Temperament*. Harper, New York.

TAYLOR, J.A. (1953) A personality scale of manifest anxiety. *Journal of Abnormal and Social Psychology*, **48**, 285.

British Journal of Dermatology (1976) 95, 379.

Hypersensitivity to bacteria in eczema

III. ARTHUS-LIKE RESPONSES TO BACTERIAL ANTIGENS WITHOUT SPECIFIC ANTIBODY

ESTHER WELBOURN, R.H.CHAMPION AND W.E.PARISH*

Department of Dermatology, Addenbrooke's Hospital, Cambridge, and
The Lister Institute of Preventive Medicine, Elstree, Herts.

Accepted for publication 10 March 1976

SUMMARY

Seven patients with a macroscopic response resembling an Arthus reaction, and occurring 2–4 h after intracutaneous injections of staphylococcal particulate antigens or total staphylococcal extract, were examined for the appropriate specific antibodies that activate complement, and by immunofluorescence for antigen, immunoglobulin and complement in the reaction site.

Four of the patients had serum antibody, and skin reactions with the typical features of early Arthus reactions and containing immunoglobulin, complement and antigen. Three patients had apparently 'non-allergic' responses. They lacked appropriate antibody in serum and immunoglobulin deposits in the lesions, though all except one had complement deposits in reaction sites. One of the three nevertheless had histological changes typical of an Arthus response. Lymphocytes teased from the reaction site skin of one patient with a 'non-allergic' 4 h response comprised 35 B cells bearing IgG out of 200 counted.

The nature of the 'non-allergic' response reaching a maximum size at about 4 h macroscopically, and in some instances microscopically resembling an Arthus response, is still to be determined but it illustrates the caution necessary in interpretation of skin tests with bacterial antigens.

We previously reported that 4 h Arthus-like skin test responses to staphylococcal and micrococcal antigens were not always related to the presence of the relevant antibacterial antibodies in the serum (Welbourn, Champion & Parish, 1976; Parish, Welbourn & Champion, 1976). Some bacterial substances have biological activities that enhance Arthus-like responses, or induce apparently antigen non-specific tissue changes that resemble allergic responses. Such changes are of clinical importance when interpreting the results of skin tests for bacterial allergy, so we further examined seven of the patients who had unequivocal reactions appearing at 2–4 h after injection of antigen.

In this preliminary examination of the problem, the results of tests for antibody were related to the findings of the intradermal skin tests and immunofluorescent examination of a sample of the skin test site. Four patients had anti-staphylococcal antibody and typical mild Arthus reactions. Another three patients did not have significant amounts of antibody, yet they had reactions macroscopically identical with those of the Arthus responders, and in one of them the histological changes were those of an Arthus response though no immunoglobulin was found in the lesion.

---

* Present address: Unilever Research, Colworth House, Sharnbrook, Bedford.

MATERIALS AND METHODS

The patients

Seven patients who had definite 2–4 h responses in previous skin tests were re-examined. The type of eczema and bacteriological findings of the seven patients were as follows.

AE. Disseminated eczema from chronic eczematous lesions on lower legs (Whitfield type). There was a sparse growth of staphylococci from the lesions. Intradermal injection of staphylococcal particulate antigen elicited an intense immediate flare which partly subsided, to be followed by a local indurated slightly congested lesion. After the skin sample was taken, there was a strong eczematous Köbner reaction at the site, and in 48 h oedema and itching with a vesicular eruption of the face and forearms, which regressed in 10 days.

BR. Severe infected eczema, with leg ulcers and phlebitis. There were numerous staphylococci in the lesions and micrococci on the normal skin.

BaP. Generalized infected eczema. He also suffers from hay fever. There were moderate numbers of staphylococci on the lesions.

CW. Disseminated infected eczema. Lesions were colonized by haemolytic Group A streptococci and *Staph. aureus*.

SH. Discoid eczema that responds to antibiotics. She also had infected teeth and throat. The episodes of tonsillitis frequently preceded exacerbation of skin lesions. There were numerous staphylococci on the lesions. After skin tests with total extract of staphylococci, she showed an intense immediate flare, followed in about 3 h by a local, indurated, mildly congested lesion.

SL. Infected chronic nummular eczema. There was a definite relation between infective episodes and exacerbation of skin lesions, and a history of chronic boils and styes. The eczematous skin and a boil yielded numerous *Staph. aureus* and diphteroids. The nose also had numerous *Staph. aureus* and micrococci.

TA. Disseminated eczema following varicose leg ulcer. A patient with auto-allergy to skin, who had formed IgG cytotoxic antibodies specific for human epidermis. Few *Staph. aureus* and no other organisms were recovered from the skin or nose. A skin test with staphylococcal total extract elicited an intense immediate flare which partially subsided to be followed 2 h later by a small indurated slightly congested reaction.

Procedures

Details of the preparation of the bacterial antigens, skin tests, immunofluorescence procedures and examination of the sera for antibodies are described or referred to in the previous reports (Welbourn *et al.*, 1976; Parish *et al.*, 1976). The Staphylococcal total extract contained 0·5 μg total substance dry weight/0·1 ml. Skin samples were taken from test sites 3–3½ h after injection of antigen.

Examination of skin lymphocytes

The dermis of a skin sample of the test site of patient TA was teased with a blunt scalpel into Eagle's medium containing 10% human serum albumin. The teased skin was then placed in 1 ml fresh medium in a narrow tube on a haematological revolving drum for 1 h. The tube was centrifuged and the cells added to those obtained by teasing. The cell suspension was passed through a column of nylon wool half an inch in length, and a suspension of small mononuclear cells presumably lymphocytes obtained. The suspension was centrifuged, and two samples of 0·1 ml prepared containing about 200 cells in each. One sample was treated with fluorescein-labelled anti-IgG, washed, and then incubated with washed normal sheep blood cells and centrifuged, to identify the B cells with IgG receptors, and T cells by the method of Papamichail *et al.* (1972). The other sample was divided into two, one of which was treated with fluorescein treated anti-IgM and the other anti-IgA.

RESULTS

Skin tests, and examination for anti-staphylococcal antibodies

The Arthus-like response was assessed by its appearance at 2–4 h after the intradermal injection, though it occurred at about 2 h in the seven patients selected as having definite reactions. It may or may not have been preceded by an immediate flare (Table 1), which partially or completely regressed before the onset of the Arthus-like response. At 2–4 h the response was circumscribed, slightly raised, firm, slightly to moderately congested, without petechiae. It persisted 4–12 h and usually regressed by 24 h.

The time of onset, the induration and congestion are typical features of Arthus responses. None of the patients showed any reaction to the control injection of diluent. Two of the seven patients showed an Arthus-like response to only one staphylococcal antigen; four patients showed responses to four or five staphylococcal or micrococcal antigens and three of these four patients also responded to the *Candida albicans* antigen (Table 1). Five patients showed a combined immediate plus Arthus-like response.

TABLE 1. Antigen non-specific and specific skin responses, and antibody titres to phenol-extracts and total extracts of *Staph. aureus* in seven eczematous patients

Patient	Atopy	Köbner	4 h* response to no/7 antigens	Staph.† antigen tested	A‡ or 1+A	Antibody titres§ PE				TE TCT
						DA	IgG	IgM	C-lysis	
AE	–	+	3	Particles	1+A	16	—	128	8	4
BR	–	–	1	Particles	A	2	—	4	0	8
BaP	+	–	6^c	Particles	1+A	2	64	—	0	0
CW	–	–	6^c	Particles	A	128	—	—	32	256
SH	–	+	5^c	TE	1+A	32	256	—	16	128
SL	–	–	1	TE	1+A	16	64	128	32	128
TA	–	–	4	TE	1+A	16	—	—	0	8

* Number of antigens to which patient gave a 4 h response. Six antigens were staphylococcal or micrococcal; those also reacting to Candida are marked c. † The antigen inducing the response examined by immunofluorescence (Table 2). ‡ A, designates Arthus-like 4 h response. 1, designates immediate (10–15 min) flare. § Antibody titres to *Staph. aureus*. PE: phenol extracted antigen. TE: total freeze-press extracted antigen. DA: direct agglutination IgG, IgM, monospecific antiglobulin titres (—, indicates no significant titre). C-lysis: complement lysis. TCT: tanned cell agglutination test.

The antibody titres to the phenol extracted antigen, and to total extracted antigen (Table 1), showed that all seven patients had some antibody to *Staph. aureus*. However, patients BR, BaP and TA appeared to have insufficient antibody to mediate an Arthus reaction in that the direct agglutination titres to both antigen preparations were 16 or less, and the antibodies did not activate complement to lyse red cells treated with the phenol extracted antigen.

Patient AE is interesting in that the antiglobulin agglutination test for IgM antibody showed a much higher titre than for direct agglutination which is unusual for tests with this antigen preparation.

Tests for Staph. Protein A, and the specificity of the anti-staph. antibodies

It was confirmed that the phenol extracted antigen did not contain staphylococcal Protein A, because 12 sera that formed good precipitation reactions in gels against staphylococcal Protein A did not do so when tested against the phenol extract. The total extract from the organism used to prepare the test antigens contained Protein A, but in small amounts when compared with the amounts extracted from cultures of other *Staph. aureus* cultures.

It was necessary to show that the antiglobulin tests for IgG anti-staphylococcal antibodies detected antigen-specific antibody combining by the Fab portion of the IgG molecule and did not reflect antigen-nonspecific binding by the Fc portion.

The IgG was separated from sera of patients SH and SL and digested with papain to obtain the Fc and Fab portions as described previously (Parish *et al.*, 1976). The Fab portion combined with red cells treated with the phenol extract and tested by anti-Fab antiserum. Pretreatment of the Fab preparation with phenol extract removed or blocked the Fab that combined with the antigen-treated red cells. This indicated that the Fab was obtained from antibody specific for the staphylococcal antigen. The Fc preparation combined in small amounts to the antigen-treated red cells as detected by anti-IgG Fc agglutination or radio-isotope binding. However, the binding by the Fc was not removed by treatment of the Fc preparation with phenol-extracted antigen. The titres obtained by anti-IgG Fc antiserum were so low (SH titre 4, SL titre 8) compared to the test antiglobulin (titres 256 and 64 respectively—Table 1) that it is unlikely that the Fc binding contributed to the agglutination in the tests on the intact sera.

Immunofluorescence tests on skin from the Arthus-like sites

Staphylococcal bacterial antigen was present in abundance at the injection sites. Most of it was in the connective tissue, and the soluble antigen tended to be adherent to the collagen fibrils of the dermis. Particulate antigen fluoresced brightly with the labelled anti-staphylococcal antibody, and could be detected within neutrophils and monocytes.

Small amounts of soluble and particulate antigen were found in perivascular lymphatics and traces of soluble antigen were present in the lumen of venules adherent to the endothelium, but it was not observed to be deposited beneath the endothelium.

Immunoglobulins were found in skin test samples of four patients, all of which contained IgG, and two also contained IgM and two IgD (Table 2). The globulins were aggregated in that they were not removed from the sections by washing for 1 hour. They appeared as fine particles, mainly perivascular, with small amounts in the perivascular lymphatic vessels. Most of it was extracellular, but IgG particles were found in neutrophils in and around vessels of patients AE, CW and SL, and IgM was found within leukocytes (unidentified) in the lumen of venules of patient AE.

One or more of the three components of complement detected by the test antiserum were found in six skin test samples. The complement components tended to be more diffuse than the immunoglobulins, and differed in distribution. They lined the lumen surface of the venular endothelium, were present in the perivascular connective tissue, though not in the perivascular lymphatic vessels, and presented as small deposits in the upper dermis. All the complement appeared to be extracellular.

Histological features

Sections of skin from five patients showed typical features of an early Arthus response (Table 2). There was swelling of the endothelium, with slight separation in samples from patients AE and SH,

TABLE 2. Findings of immunofluorescence tests of samples taken from the Arthus-like reactions 2–3 h after injection of the antigen, and histological resemblance to an Arthus response

Patient	Staph. antigen tested	Immunofluorescent detection of						Histological resemblance to Arthus
		Immunoglobulins				Complement	Antigen	
		IgG	IgM	IgD	IgE	C3c, 3d, 4		
AE	Particles	+	+	−	−	+	+	Yes
BR	Particles	−	−	−	−	+	+	No
BaP	Particles	−	−	−	−	+	+	Yes
CW	Particles	+	−	+	−	+	+	Yes
SH	TE	+	−	−	−	+	+	Yes
SL	TE	+	+	+	−	+	+	Yes
TA	TE	−	−	−	−	−	+	No

perivascular oedema, diapedesis of red cells in patient SL but insufficient to be termed a petechial haemorrhage, a moderate neutrophilia within and around the blood vessels (Figs 1, 2), and in patients AE, BaP, SH and SL granular material on the endothelium that stained by the Feulgen technique and probably represented nuclear debris from degenerate leukocytes. The neutrophilia was greater in persons injected with particulate than with soluble antigen. A few eosinophils were seen in the sections of patient BaP.

Patients BR and TA did not show features of a typical Arthus reaction. Though there were numerous neutrophils lining the lumen surface of the blood vessel endothelium in the sample from BR, the endothelium appeared to be intact. There was a sparse infiltration by neutrophils and small mono-

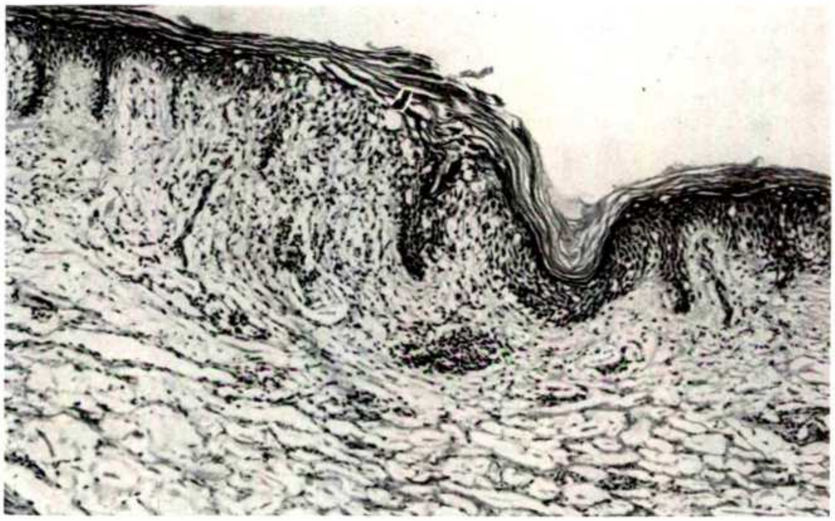


FIGURE 1. Reaction site from patient BaP 3·5 h after injection of staphylococcal particulate antigen. There is mild leucocytoclastic angiitis with neutrophilia round vessels and sparse to moderate infiltration of the dermis. Other changes are oedema of the dermis and vacuolation of epidermis. This patient had very little anti-staphylococcal antibody in the serum; complement but no immunoglobulin was found in the lesion (H & E, × 75).

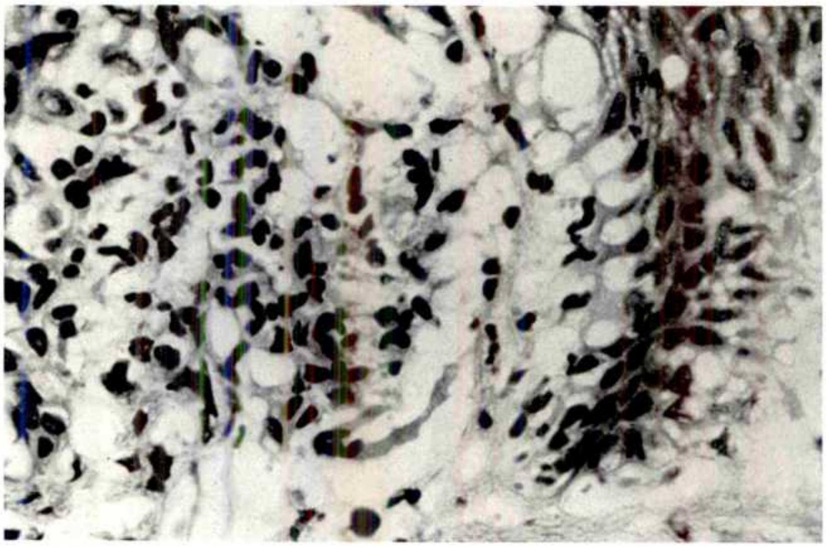


FIGURE 2. Greater magnification of a field of the reaction site in Fig. 1 showing neutrophils emigrating from a damaged venule in the upper dermis, and oedema of the dermal papilla extending to the dermo–epidermal junction (H & E, × 350).

nuclear cells in a ratio of about 3:1 around both venules and arterioles of the upper dermis, and spreading diffusely towards the dermo–epidermal junction. Similar changes were seen in the sample from patient TA, injected with soluble antigen, but there were fewer neutrophils within the vessels, and all appeared intact. The mononuclear cells were considered to be lymphocytes.

Examination of the skin lymphocytes of patient TA

In a sample of about 200 small mononuclear cells teased from the skin samples of patient TA and treated with fluorescein-labelled anti-IgG, followed by incubation with washed normal sheep erythrocytes, 35 cells stained for IgG and were presumably B-lymphocytes, 18 formed rosettes with three or more sheep red cells and were presumably T-lymphocytes. Two of the 18 cells forming rosettes also stained weakly with the anti-IgG antiserum.

Too few cells were recovered after treatment of the other portions of the cell suspension to detect IgM or IgA on the cells.

DISCUSSION

The main features of the Arthus response are the formation of complexes of precipitating antibody with antigen which is in a slight to moderate excess of equivalence to the antibody. The complexes must activate complement and be deposited beneath the vascular endothelium. Neutrophils, attracted to the complexes by complement components, ingest the complexes and while degrading them release lysosomal proteolytic enzymes. The free enzymes degrade the internal elastic lamina of larger and the basement membrane of smaller vessels. This enhances neutrophil emigration and further tissue change as long as the antigen–antibody complexes persist to attract further neutrophils. When the complexes are degraded, mononuclear cells infiltrate the site and macrophages remove the cell debris as resolution occurs (Cochrane, 1967).

The response is modified by the size of the complexes depending upon the molecular weight of the antigen, the immunoglobulin class and avidity of the antibody, and proportion of antigen to antibody molecules. Other conditions may enhance Arthus reactions. Certain bacterial substances, e.g. lipoteichoic acids or lipopolysaccharides, as antigens increase the severity of the response compared to bacterial or bland proteins (Parish & Rhodes, 1967; Parish, 1971 and to be published).

The typical macroscopic appearance of an Arthus response usually reaching its maximum size at four hours is a circumscribed, raised indurated lesion, slightly oedematous, congested and scattered with petechiae. In mild responses there is less oedema, and the congestion is not accompanied by haemorrhage. In severe reactions, the lesion is larger, with more diffuse oedema, and a central haemorrhage that may progress to a scab and slough.

The main histological changes at about 4 h are the intense neutrophil infiltration of affected vessels and perivascular tissue, with some more generalized neutrophilia. There is degeneration of the endothelium and degradation of the vessel wall, particularly in venules, and formation of leukocyte–platelet thrombi, sometimes accompanied by fibrinoid change. There may be haemorrhage according to the severity, and there is always congestion and oedema towards the periphery.

The infiltrating neutrophils degrade the antibody and complement of the complexes within 6–8 h. However, in most lesions, except the mildest, it is usual to detect immunoglobulin, some complement components and the injected antigen by immunofluorescence at 2–4 h after eliciting the reaction.

Previously we reported that in patients injected intradermally with antigens of *Staph. aureus* or micrococcus (Baird-Parker types S1 and M1 respectively), twenty-six persons with an Arthus-like response had what were considered to be appropriate amounts of complement-activating antibody in the serum and twenty-seven persons did not (Parish *et al.*, 1976). We now report the results of more detailed tests on seven patients, which are summarized in Table 3. The seven patients may be considered in two groups. In one group are four patients with allergic Arthus responses. In the other group are three patients with apparently 'non-allergic' 4 h lesions that macroscopically resembled Arthus responses. The macroscopic changes at the skin test sites appeared to be identical in all seven patients, varying a little in severity.

Four of the patients appeared to have typical mild Arthus responses, with sufficient complement-activating antibody in the serum, and characteristic though mild histological changes in the lesions, which contained immunoglobulin and complement when examined by immunofluorescence. These four patients could be considered to be positive controls, though not intended as such.

The other three of the seven patients did not have complement-activating anti-staphylococcal antibody and there were no deposits of immunoglobulin in the reaction sites. The correlation between serum antibody and immunoglobulin deposited in the lesions was good, though complement was found

TABLE 3. Summary of findings to identify the reactions as Arthus responses

Patient	Specific Ab with C-lysis	Immunofluorescence immunoglobulin	Histology Arthus-like	Arthus response
AE	+	+	+	Yes
BR	−	−	−	No
BaP	−	−	+	No
CW	+	+	+	Yes
SH	+	+	+	Yes
SL	+	+	+	Yes
TA	−	−	−	No

in samples of two patients in the absence of immunoglobulin (Table 2). In these two samples the complement may have participated as an acute phase inflammatory protein, or was activated by the bacterial antigen in the absence of specific antibody.

Furthermore despite the similarity of the macroscopic reactions, the histological changes in two of the three 'non-allergic' lesions did not resemble a true Arthus response. The histological changes of one patient (BaP) proved to be the one exception in the group because, though there was no specific antibody and no immunoglobulin in the test site, the histology resembled that of an Arthus response.

It would be inappropriate to discuss the results of this preliminary investigation in detail, but the following are possible explanations for the occurrence of 'non-allergic' 4 h skin test responses to bacterial antigens.

The agglutination and complement lysis tests do not detect all the anti-staphylococcal antibody, even though total extracts were used as antigen in the tanned cell tests. Antibody to staphylococcal teichoic acids has been detected in precipitation tests using whole sera of staphylococcal infected persons or concentrated gamma globulins of normal persons (Daugharty, Martin & White, 1967). Agglutination tests are usually more discriminating than the precipitation technique, and there is good evidence that the phenol extracted antigen is mainly teichoic acid (Parish *et al.*, 1976), yet very small amounts of antibody were found in the sera of some persons, eczematous or normal.

It is possible that bacteria contain substances that activate complement and induce inflammatory change in the absence of specific antibody. The lipopolysaccharide of Gram negative organisms has this property. So has C-substance, in several bacteria and fungi, which combines with C-reactive protein in human sera. *Staphylococcus aureus* forms a substance, Protein A, that binds with the Fc portion of IgG and induces a reaction resembling an Arthus response in man and animals (Forsgren & Sjöquist 1966; Gustafson, Sjöquist & Stalenhein, 1967; Martin, Crowder & White, 1967). Protein A is not thought to have elicited the reactions we observed, because the antigens containing it should have elicited reactions in all persons tested. Furthermore, in our previous tests on the greater number of patients, some persons without specific antibody showed Arthus-like responses to micrococci and to staphylococcal phenol extracts which did not contain Protein A. However Protein A or similar substances occur in other bacteria, e.g. streptococci and pneumococci (Kronvall, 1973; Christensen & Kronvall, 1974; Stephens *et al.*, 1974) and should always be considered as a possible cause of 4 h reactions in skin tests.

The finding of a high proportion of B lymphocytes with IgG determinants in the skin test site of patient TA is particularly interesting, and other examples of this phenomenon will be described elsewhere. This patient had a typical 4 h Arthus-like response in the absence of anti-staphylococcal circulating antibody, or deposits of immunoglobulin in the lesion. The lymphocytes may have contributed to the reaction.

In further studies on the nature of 4 h Arthus-like reactions apparently occurring in the absence of specific antibody, it will be essential to use pure preparations of single bacterial antigens, but there is sufficient evidence that such reactions occur, and may give rise to error when interpreting the results of skin tests.

ACKNOWLEDGMENTS

We are grateful to the Smith Kline & French Foundation for research grants to E.W. and W.E.P., and also to Addenbrooke's Hospital Research Fund for a further grant to E.W., without whose help this and related long-term studies could not have been completed.

We are grateful for advice and support from Drs Arthur Rook and Granville R.E.Naylor, and also to Drs Arthur Rook and Stephen Roberts for referring patients for the investigation. We thank Dr

M.H.Gleeson-White, Mr S.T.Haslam and the Bacteriological Laboratory for the identification of the bacteriological cultures, and the following staff of the Lister Institute for much careful technical assistance—Miss Marisa Walisiewick, Mrs Gillian Wardle, Mrs Geraldine Yates, Mr Kenneth Veal, Mr Michael Scott and Miss Susan Hedges. We thank the Junior Staff of the Dermatology Department, Addenbrooke's Hospital for their help, and we especially thank the patients and the controls for their co-operation for so many years.

REFERENCES

CHRISTENSEN, P. & KRONVALL, G. (1974) Capacity of Group A, B, C, D and G streptococci to agglutinate sensitized sheep red cells. *Acta pathologica et microbiologica Scandinavica, Section B*, **82**, 19.

COCHRANE, C.G. (1967) Mediators of the Arthus and related reactions. *Progress in Allergy*, **11**, 1.

DAUGHARTY, H., MARTIN, R.R. & WHITE, A. (1967) Antibodies against staphylococcal teichoic acids and type-specific antigens in man. *Journal of Immunology*, **98**, 1123.

FORSGREN, A. & SJÖQUIST, J. (1966) Protein A from *S. aureus*. I. Pseudoimmune reaction with human gamma-globulin. *Journal of Immunology*, **97**, 822.

GUSTAFSON, G.T., SJÖQUIST, J. & STALENHEIN, G. (1967) 'Protein A' from *Staphylococcus aureus*. II. Arthus-like reaction produced in rabbits by interaction of protein A and human γ globulin. *Journal of Immunology*, **98**, 1178.

KRONVALL, G. (1973) A surface component in Group A, C and G streptococci with non-immune reactivity for immunoglobulin G. *Journal of Immunology*, **111**, 1401.

MARTIN, R.R., CROWDER, J.G. & WHITE, A. (1967) Human reaction to staphylococcal antigens. A possible role of leukocyte lysosomal enzymes. *Journal of Immunology*, **99**, 269.

PAPAMICHAIL, M., HOLBOROW, E.J., KEITH, H.I. & CURREY, H.L.E. (1972) Subpopulations of human peripheral blood lymphocytes distinguished by combined rosette formation and membrane immunofluorescence. *Lancet*, **ii**, 64.

PARISH, W.E. (1971) Studies on vasculitis. I. Immunoglobulins, β_1C, C-reactive protein and bacterial antigens in cutaneous vasculitis lesions. *Clinical Allergy*, **1**, 97.

PARISH, W.E. & RHODES, E.L. (1967) Bacterial antigens and aggregated gamma globulin in the lesions of nodular vasculitis. *British Journal of Dermatology* **79**, 131.

PARISH, W.E., WELBOURN, E. & CHAMPION, R.H. (1976) Hypersensitivity to bacteria in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci. *British Journal of Dermatology*, **95**, 285.

STEPHENS, C.G., REED, W.P., KRONVALL, G. & WILLIAMS, R.C. (1974) Reaction between certain strains of pneumococci and Fc of IgGl. *Journal of Immunology*, **112**, 1955.

WELBOURN, E., CHAMPION, R.H. & PARISH, W.E. (1976) Hypersensitivity to bacteria in eczema. I. Bacterial culture, skin tests, and immunofluorescent detection of immunoglobulins and bacterial antigens. *British Journal of Dermatology*, **94**, 619.

British Journal of Dermatology (1976) **95**, 389.

Pigmented lesions in newborn infants

ROBERT G.WALTON, ALVIN H.JACOBS AND ALVIN J.COX

Stanford University School of Medicine, Stanford, California, U.S.A.

Accepted for publication 26 March 1976

SUMMARY

1058 newborn infants were examined. Forty-one (3·9%) had clinically discernible pigmented lesions compatible with melanocytic naevi. Biopsy was performed on thirty-four of the forty-one and of these; eleven, representing 1·01% of the infants, proved to be melanocytic naevi. No giant (garment) naevi were seen in this series. Two of the eleven naevi pathologically examined showed histological changes similar to those that have been reported in some giant naevi, but the remaining nine were not only different from criteria usually assigned to giant naevi, but they also differed from the usual adult naevi, in that most were predominantly junctional. None of the melanocytic naevi in this series showed any suggestion of malignant change. In newborn infants it is often impossible clinically to distinguish naevi from other types of pigmented lesions, as only eleven out of the thirty-four pigmented lesions were melanocytic naevi. Seven of the eleven melanocytic naevi were under 1·5 cm in diameter. No pigmented lesions were found on the palms, soles or genitalia.

The purpose of this study is to evaluate, clinically and pathologically, pigmented lesions in newborn babies with a sufficient number of infants and lesions to be statistically relevant. Not all pigmented lesions in newborn infants are melanocytic naevi (naevus cell naevi). Some congenital naevi are clinically and pathologically of a type that has been reported as having a much higher incidence of malignant degeneration than do acquired naevi. It presently appears that it is the so-called giant or garment type of naevus that is more often associated with malignancy.

A careful review of the world literature shows no such reported study. Although a number of pertinent and significant findings with respect to pigmented lesions and melanocytic naevi in the newborn have been documented, there are no accurate records with regard to the incidence of clinical and pathological types.

Pratt (1953) examined 1096 newborn infants for pigmented naevi and these were described as distinctly light brown, well marginated macules from 0·5 to 1 cm in diameter; 879 of the newborns were white and 2·7% of these had clinically diagnosed pigmented lesions. Two hundred and seventeen were black babies and 15·6% of these were thought to have pigmented naevi. No biopsies were performed.

Reprint requests to: Dr A.H.Jacobs, Department of Dermatology, Stanford University Medical Center, Stanford, California 94305, U.S.A.

In the examination of 200 infants, Pack & Davis (1956) found an incidence of only 2·4% of pigmented naevi, but again the results of biopsies, if performed, were not documented. Reed *et al.* (1965) reported on fifty-five patients with giant garment pigmented naevi with special attention to the clinical features of the naevi and to their histopathological characteristics. They also reviewed 200 patients in the literature with giant naevi, presumably congenital, and found that the incidence of malignancy in different series varies from 1·8 to 13% and they emphasized the importance of the incidence of malignancy in congenital naevi. Sanderson (1972) stated that the important difference between naevi

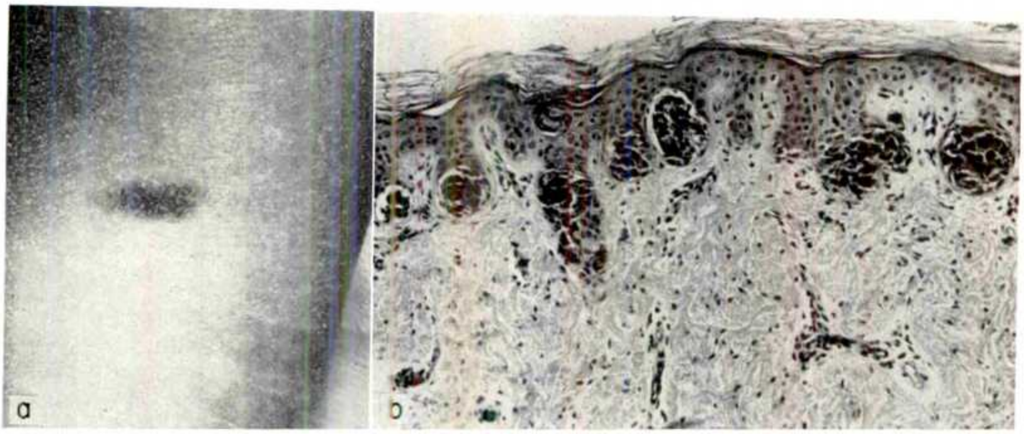


FIGURE 1. (a) Pigmented macule measuring 0·6 × 0·35 cm on the right posterior leg of a newborn infant of Mexican–American parentage.
(b) Naevus cells, which are heavily pigmented, lie in prominent groups, entirely within the epidermis forming an intra-epidermal naevus (H & E, × 150).

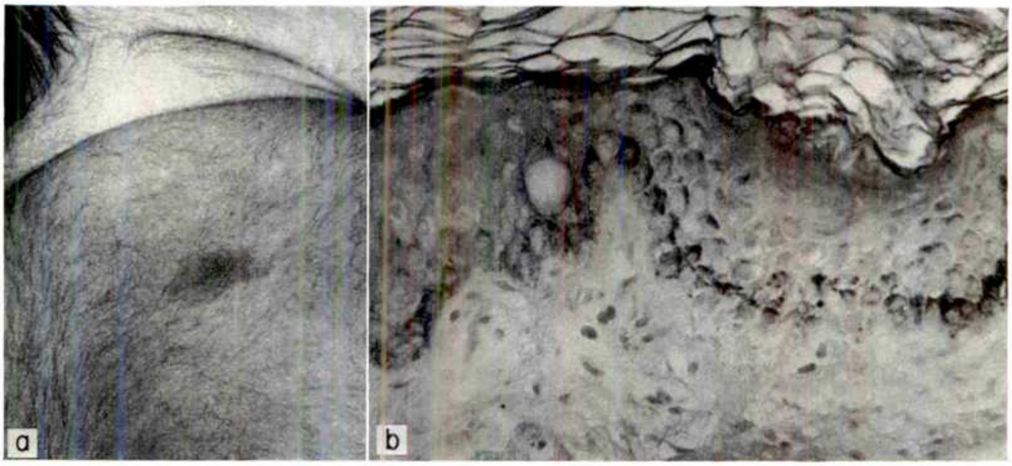


FIGURE 2. (a) Light brown macule measuring 1·7 × 0·6 cm on the upper back of a polynesian newborn.
(b) There are no naevus cells. Increased amounts of melanin are concentrated irregularly in the basal cells of the epidermis. There are no epidermal changes of lentigo so this is regarded as a café-au-lait spot (Ferric Ferricyanide technique of Schmorl, × 450).

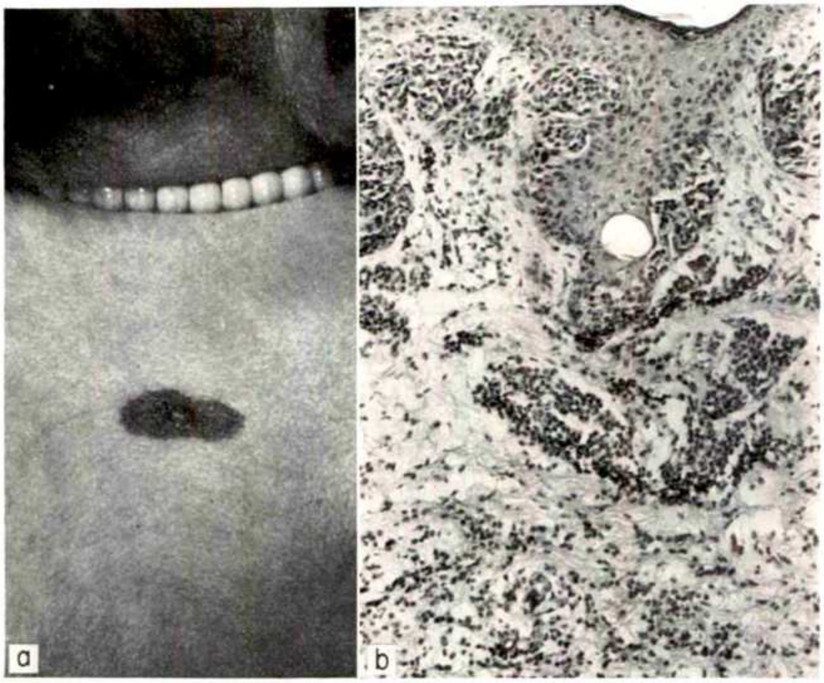


FIGURE 3. (a) Darkly pigmented macule, measuring 1·0 × 0·5 cm on the back. The dark area centrally represents the biopsy site wound.

(b) In addition to prominent naevus cell clusters within the epidermis and in the superficial dermis, there is a more diffuse spread of naevus cells in the deep half of the dermis. This is one of two examples of this pattern (H & E, × 250).

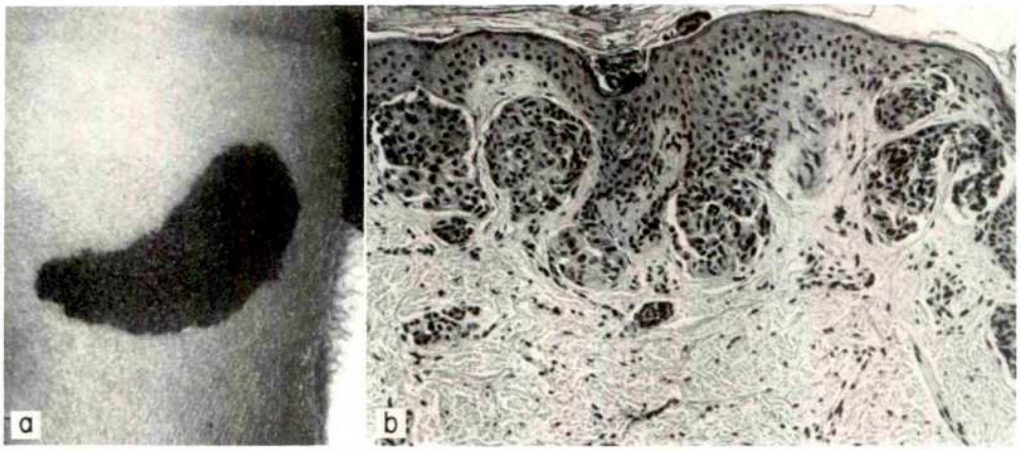


FIGURE 4. (a) Pigmented macule measuring 3·0 × 1·5 cm on the posterior thigh. This was the largest naevus in the study.

(b) This compound naevus resembles that of Case 3b. The histological appearance provides no indication that this was a large lesion (H & E, × 150).

present at birth and those that arise later is that the congenital ones are usually more extensive and have a tendency to malignant change (10% in one recent series: Greeley, Middleton & Curtin, 1965). Any over 1·5 cm in diameter are likely to have been present at birth and have distinctive characteristics. Mark *et al.* (1973) reported on small and garment types of melanocytic naevi that were stated to have been congenital. Most of these lesions were not examined in the newborn period and the basis for considering them congenital was historical. The clinical and histological features of sixty congenital, compound and dermal naevi were reviewed and compared to those of sixty acquired, compound and dermal naevi. Clinically, the two groups could be distinguished by size, most congenital naevi being larger than 1·5 cm and most acquired naevi smaller than 1·5 cm in diameter. They stated that congenital naevi commonly are different in colour and surface topography from acquired naevi. They also listed histological differences between congenital naevi and acquired naevi. In contradistinction to acquired naevi that seemed to vary in their characteristics in different age groups, congenital naevi had similar histological characteristics regardless of age at excision. It may be pertinent that most of their naevi were in adults. Because of the known association of congenital garment naevi with malignant melanoma, these authors felt it was of importance to compare the histology of garment naevi with other small congenital naevi. They found that the histological picture in all cases of congenital naevi, irrespective of size, was similar.

METHOD AND MATERIALS

Of 1058 unselected newborn babies, 550 were examined at Doctors Hospital, Modesto, California (Walton) and 503 were examined at Stanford University Hospital, Stanford, California (Jacobs). All babies included were born in the institutions named. No baby was older than 72 h and the vast majority were under 48 h. The entire skin surface was examined, including the lips, perianal area, genitalia, eyelids, palms, soles, scalp and nails. However, the mouth was not examined and in uncircumcised males, the prepuce was not retracted and therefore the glans penis was not examined. All cutaneous birthmarks, tumours and defects were noted, but only the pigmented lesions will be reported in detail in this paper; the subject of other birthmarks in the newborn will be presented elsewhere (Jacobs & Walton, 1976). All pigmented lesions were noted regarding location and morphology. Colour photographs were taken and a 2 mm punch biopsy was performed from each lesion, except for seven where biopsy was refused.*

Of the 1058 infants examined, 841 (79·5%) were white, 66 (6·2%) were black, 118 (11·2%) Mexican-Americans, 27 (2·6%) Oriental and 6 (0·6%) represented other racial groups. Five hundred and sixty-one of the babies were male and 497 were female.

RESULTS

The following are the results of the examination of pigmented lesions in 1058 infants, excluding the obvious mongolian spots. Forty-one (3·9%) of the infants had clinically recognizable pigmented lesions, the majority of which had a gross appearance of melanocytic naevi. In seven, biopsy was refused. Therefore, there was a total of thirty-four pigmented lesions biopsied. The pigmented lesions were racially distributed as follows: twenty-one (2·5%) of 341 were in white infants; thirteen (19·7%) of sixty-six were in blacks (but in five of these biopsy was refused); four (3·4%) out of 118 in Mexican-American babies (but in one biopsy was refused); one (3·7%) of twenty-seven Orientals and two out of six (33·3%) in other racial groups (biopsy was refused in one of these).

---

*The protocol for this study was approved by the Human Research Committee of Stanford University and informed parental consent was obtained

TABLE 1. In 1058 newborn infants examined, forty-two had pigmented lesions: thirty-four of these were biopsied

Intra-epidermal naevi	3
*Compound naevi	8
Lentigo	4
Café-au-lait	5
Fibrosis and increased capillaries	8
Toxic erythema of newborn	2
Mongolian spot	1
Naevus sebaceous	1
Leiomyoma	1
No identifiable disease	1

*All but two of what are called compound naevi were superficial and resembled the intra-epidermal group. Both of these categories are sometimes referred to as junctional naevi.

TABLE 2. Melanocytic naevi at birth eleven of 1058 or (1·01%)

	Race	Sex	Location	Size (cm)	*Path
1.	Caucasian	F	Back	1·5 × 0·5	CN
2.	Caucasian	M	Back	1·0 × 0·5	CN
3.	Caucasian	M	Ext. forearm	0·25 × 0·25	CN
4.	Caucasian	M	Post. thigh	3·0 × 1·5	CN
5.	Caucasian	M	Right knee	0·5 × 0·5	CN
6.	Caucasian	F	Left buttock	0·2 × 0·2	JN (IEN)
7.	Mexican	M	Rt. lower leg	0·6 × 0·35	JN (IEN)
8.	Caucasian	F	Left knee	2·0 × 2·7	JN (IEN)
9.	Black	F	Right buttock	1·6 × 0·4	CN
10.	Black	M	Scalp	1·6 × 0·9	CN
11.	Oriental	M	Left ant. chest	0·7 × 0·3	CN

* CN, compound naevus; IEN, intra-epidermal naevus.

Of the thirty-four pigmented lesions biopsied, eleven (1·01% of 1058 babies) proved to be melanocytic naevi. In this series none was categorized as a garment or giant naevus. Seven of the eleven pathologically proved melanocytic naevi were less than 1·5 cm in diameter. None of the naevi was located on the hands, feet or genitalia. Table 1 illustrates the histological diagnosis of the 34 biopsied pigmented lesions. Table 2 shows the racial origin, sex, location, size and type of the eleven melanocytic naevi.

Histologically, all but two of the naevi presented a similar pattern. The naevus cells formed well-defined, rounded groups lying within the base of the epidermis in the pattern of junctional naevi. In six of the lesions there were similar naevus cell clusters in the papillary or upper reticular dermis. Three of these lesions in which the naevus cells were entirely intra-epidermal did not appear to represent a distinctly different group. In three instances some of the naevus cells were clearly related to the distal portions of hair follicles. There was no diffuse growth of naevus cells or extension to the deep dermis.

Two of the naevi presented a more diffuse permeation of the dermal connective tissue as far as the lower reticular dermis by naevus cells. We were not able to identify prominent specific involvement of the deep appendages, nerves or vessels in the pattern described by Mark *et al.* (1973) as characteristic of congenital naevi, although in one of the lesions there was a focus of naevus cells adjacent to a sweat duct. It should be noted, however, that the specimens were small and contained only a few adnexal structures.

Of interest is the observation that in spite of the frequency of intra-epidermal naevus cell groups in our congenital naevi and of the presence of prominent pigmentation of basal keratinocytes associated with several of the naevi, there was very little prominence of non-nested basal epidermal clear cells resembling melanocytes. These cells are frequently numerous in naevi of adults and they are listed by Mark *et al.* (1973) as a feature of both congenital and acquired naevi.

The histological interpretation of the twenty-three biopsied pigmented lesions other than naevi is as follows: four have been classified as lentigo by virtue of prominent epidermal pigment, hyperplasia of the epidermis and the presence of prominent clear cells at the epidermal base. Five exhibited increased melanin in the base of the epidermis without epidermal hyperplasia and these have been classified as café-au-lait spots. Two lesions showed mild irregular deposits of epidermal melanin associated with dermal changes; these have been regarded as post-inflammatory pigmentation and are included in the next group. Eight speciments showed more than average prominence of fibroblasts and capillaries in the upper dermis. Most of these exhibited little or no recognizable melanin. In two specimens, telangiectasia was conspicuous, and this is regarded as the probable cause of the gross appearance of the subpapillary dermis, suggesting oedema, and in some, this zone gave a positive reaction for acid mucopolysaccharide with the colloidal iron technique. Several of these specimens showed a very mild infiltration of lymphoid cells in the deeper dermis. Whether these changes represent instances of pathological inflammation has not been determined. Mast cells were slightly increased in several of the specimens, but to no greater degree than can be explained by a mild reactive process, so it has been concluded that these lesions do not represent urticaria pigmentosa. In two instances there was more pronounced diffuse infiltration of inflammatory cells throughout the dermis. These included lymphoid cells, neutrophilic leukocytes and prominent eosinophils. These cases are regarded as instances of so called toxic erythema of the newborn. One lesion histologically was typical of a mongolian spot, although clinically had been diagnosed as a possible melanocytic naevus ot the arm. There were single instances of naevus sebaceus and of dermal leiomyoma. One specimen showed no recognizable abnormality. There were two pigmented lesions biopsied in one baby. One of the lesions from the lower leg was fairly large, measuring 1·5 × 1·5 cm and showed chronic inflammation and melanin pigmentation, whereas the other from the right thumb was 0·2 cm in diameter and showed intra-corneal haemorrhage and chronic inflammation. We believe this to be the result of intra-uterine thumb sucking.

The grouping of lesions characterized by increased epidermal melanin but no naevus cells is arbitrary, since fully accurate categorization of such pigmented spots is not possible. The distinctly irregular accumulation of melanin in several lesions calls attention to the possibility that some of the pigmented spots may represent post-inflammatory pigmentation.

DISCUSSION

This study differs from previously reported series by virtue of the examination of all cutaneous lesions within the newborn period. It has been postulated that there is a life cycle of the melanocytic naevus, whereby a juvenile lentigo occurs in infancy or at birth and develops into a junctional naevus, later into a compound naevus and finally into an intradermal naevus. In our study, four of the thirty-four pathologically examined lesions were classified as lentigo and nine of the naevi were of the junctional type of which only three were entirely intraepidermal. The remaining two were compound naevi with a predominantly dermal component and therefore our findings correspond to the usually accepted natural history of naevi. It is possible that congenital naevi and acquired naevi may have different life cycles or even different dynamic functions, or that the aging process of some naevi may be accelerated *in utero*. In any event, no naevus was entirely intradermal, and none of the eleven naevi were quite superficial and did differ from most naevi of adults, so it is possible that there are maturation changes in such lesions both before and after birth. This might account for the differences between our findings and those of Mark *et al.* (1973) and of others who have examined congenital naevi after infancy has passed. It should be emphasized that our study is entirely on newborn babies, whereas they based their conclusions on naevi in older subjects and the origin of the naevi was diagnosed by history. Possibly it is only the larger naevi, present at birth, that are noticed or remembered as such in adult life, whereas small pigmented lesions may go unnoticed at birth or be forgotten, and later misdiagnosed as acquired naevi.

Previous documentation suggests that many congenital naevi have a particular histological picture which is associated with an increased malignant degeneration. Only two of the eleven melanocytic naevi reported here can in any way be fitted into this category. The majority of the melanocytic naevi in this series are small, under 1·5 cm in diameter; this is also in contrast to previous findings (Mark *et al.*, 1973; Sanderson, 1972). None of the naevi was of the giant garment type, although one was fairly large situated on the posterior thigh and measured 3·0 × 1·5 cm. The number of black babies examined was too few to be of statistical significance. Our findings give the impression that there is a higher incidence of pigmented lesions in black babies. A further study on black babies is being carried out (Naness *et al.*, 1976).

It should be emphasized that clinically there was no way of knowing when a pigmented lesion was a naevus of the junctional type or of the compound type, or indeed whether a pigmented lesion was in fact a melanocytic naevus. Pathological examination was the only way that this could be accurately determined. It is recognized that the 2 mm punch biopsy is small and may not in all cases present sufficient representative tissue to be completely diagnostic. Since the specimens were removed from the centre of the lesions, however, it is unlikely that they failed to identify any melanocytic naevi or provide a representative appearance. Certainly all melanocytic naevi at birth are not large, as defined by a diameter of at least 1·5 cm and we are not sure that all large melanocytic naevi have been present at birth, or whether some large naevi are acquired later in life. In fact, recent unpublished studies of ours have shown that naevi over 1·5 cm in diameter can be acquired naevi.

The findings of this study, indicating an incidence of melanocytic naevi in more than 1% of newborn infants, together with the rarity of death from melanoma arising in a congenital naevus, indicate that the great majority of congenital naevi do not develop into melanoma. It is probable that most malignancy in congenital naevi appears in the garment type, which is sufficiently uncommon that no such case was included in this series. Our experience contradicts the statements of Sanderson (1972) and of Mark *et al.* (1973) that congenital naevi are usually larger than those appearing later in life. We believe that the principal reason for this discrepancy is that the lesions of our study represent the actual incidence of naevi present at birth, while studies based upon a history of the presence of lesions at birth can be misleading because congenital naevi are usually larger than those appearing later in life.

ACKNOWLEDGMENTS

The authors wish to express their thanks to the medical and nursing staffs of the newborn services at Doctors Hospital in Modesto, California and at Stanford University Hospital, Stanford, California for their co-operation.

The portion of this investigation carried out at Stanford University Medical Center was supported in part by a grant (RR-0081) from the General Clinical Research Centers Program of the Division of Research Resources National Institutes of Health.

REFERENCES

GREELEY, P.W., MIDDLETON, A.G. & CURTIN, J.W. (1965) Incidence of malignancy in giant pigmented naevi. *Reconstructive Surgery*, 36, 26.

JACOBS, A.H. & WALTON, R.G. (1976) Incidence of birthmarks in the neonate. *Pediatrics*, in press.

MARK, G.J., MIHN, M.C., LITEPLO, M.G., REED, R.J. & CLARK, W.H. (1973) Congenital melanocytic naevi of the small and garment type. *Human Pathology*, 4, 395.

NANESS, J., WALTON, R.G., GOTTLIEB, B. & CASTELLANO, A. (1976) *Pigmented naevus study of newborns*, to be published.

PACK, G.T. & DAVIS, J. (1956) Moles. *New York State Journal of Medicine*, 56, 3498.

PRATT, A.G. (1953) Birthmarks in infants. *Archives of Dermatology*, 67, 302.

REED, W.B., BECKER, S.W., SR., BECKER, S.W., JR. & NICKEL, W.R. (1965) Giant pigmented naevi, melanoma and leptomeningeal melanocytosis. *Archives of Dermatology*, 91, 100.

SANDERSON, K.V. (1972) Naevi and other developmental defects. *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling) 2nd edn, Vol. 1, p. 163. Blackwell Scientific Publications, Oxford.

British Journal of Dermatology (1976) **95**, 397.

The effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins

JONATHAN T. LORD, VINCENT A. ZIBOH,* JOSEPH POITIER,
GERALD LEGGET AND NEAL S.PENNEYS

Departments of Dermatology and Biochemistry,
University of Miami School of Medicine, Miami, Florida 33152, U.S.A.

Accepted for publication 12 March 1976

SUMMARY

Prostaglandin biosynthesis from arachidonic acid by skin microsomal fraction preparation was enhanced by UV-irradiation at wavelengths of 254 and 360 nm. In the presence of 8-methoxy psoralen (8 MOP) and coal tar, prostaglandin biosynthesis was further enhanced approximately 2-fold by UV-irradiation at 254 nm. Stimulation was less by UV-irradiation at 360 nm.

8-MOP enhanced the conversion of PGE_2 into $PGF_{2\alpha}$ by PGE_2-9-ketoreductase prepared from skin high speed supernatant fractions. UV-irradiation at 254 nm and 360 nm with or without the photosensitizers had no effect on the activity of the PGE_2-9-ketoreductase.

These data therefore indicate that the action of UV-irradiation, 8-methoxy psoralen and coal tar on the skin may in part be due to their regulation of the biosynthesis and metabolism of prostaglandins in this tissue.

The prostaglandins constitute a class of compounds that are produced by all cells in the mammalian body from the essential fatty acids. The biosynthesis of prostaglandins from arachidonic acid by human and rat skin has been reported (Ziboh & Hsia, 1971; Van Dorp, 1971; Jonsson & Angaard, 1972; Greaves & McDonald, 1972). Ziboh reported that the enzyme prostaglandin synthetase is located in the microsomal fraction of the human epidermis (Ziboh, 1973). There is also evidence that the skin contains enzyme systems for the metabolism of prostaglandins (Jonsson & Angaard, 1972; Ziboh, Lord & Penneys, 1975). The conversion of prostaglandin E_2 (PGE_2) into prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$) in human and rat skin specimens has also been described recently (Ziboh *et al.*, 1975), indicating the presence of the enzyme PGE_2-9-ketoreductase in this tissue. When skin was exposed to ultraviolet (UV)-irradiation, prostaglandin-like compounds were detected in increased concentrations in areas of delayed inflammation (Mathur & Gandhi, 1972; Greaves & Sondergaard, 1970). Topical

---

* Correspondence to: Vincent A. Ziboh, PhD, Department of Dermatology, University of Miami School of Medicine, P.O. Box 520875, Biscayne Annex, Miami, Fla. 33152, U.S.A.

application of indomethacin to skin prior to exposure of ultraviolet irradiation resulted in the suppression of erythema (Snyder & Eaglstein, 1974). Furthermore, intradermal injection of PGE_2 caused erythema, oedema and tenderness (Sondergaard, Pekka & Jorgensen, 1973). The above reports suggest in part that a relationship exists between UV-irradiation, the release of prostaglandins and the development of sunburn erythema in the skin.

Since prostaglandin synthesis is induced in skin by UV-irradiation it seems reasonable to speculate that photosensitizing compounds may also play a role in the biosynthesis and metabolism of prostaglandins. Current therapies for the management of psoriasis employ compounds that are photoreactive with UV-irradiation of varying wavelengths (Pathak, 1961; Goeckerman, 1931). The clinical manifestation common to these treatments is the production of erythema. One compound, 8-methoxy psoralen, is used in conjunction with long wave UV-irradiation (360 nm), a wavelength that by itself will not produce erythema. However, erythema is noted after UV-irradiation and a systemic dose of psoralens (Pathak, 1961). Patients treated with psoralens are very sensitive to sunlight exposure for at least 8 h after treatment, because severe erythema is produced (Fitzpatrick *et al.*, 1955).

In this study, we report (i) the effects of 8-methoxy psoralen, coal tar and mycophenolic acid on the biosynthesis and metabolism of prostaglandins; (ii) the effect of UV-irradiation and the above substances on the enzyme systems involved with the biosynthesis and metabolism of prostaglandins.

MATERIALS AND METHODS

[1-^{14}C]Arachidonic acid (specific radiopurity, 58·0 mCi/mmole) and ^{3}H-PGE_2 (specific activity 125 mCi/mole) were purchased from New England Nuclear, Boston, Mass., U.S.A. Radiopurity was ascertained by TLC on silica gel G in solvent system chloroform–methanol–acetic acid (90:5:5, by vol). PGE_2 and $PGF_{2\alpha}$ were gifts from Dr U.F.Axen of the Upjohn Co., Kalamazoo, Michigan, U.S.A. Non-radioactive arachidonic acid (99% purity) was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. The 8-methoxy psoralen used in the experiments was a product of the Elder Co., Bryan, Ohio, U.S.A. Purity was determined by TLC on silica gel G plates. Coal tar was a gift from the Westwood Pharmaceutical Co., Buffalo, N.Y., U.S.A. Reagents were of analytical grade and solvents were redistilled before use. UV-irradiation sources used were a Universal Model UVS-54 (energy $1{\cdot}4 \times 10^{-3}$ j/cm^2/s) for 254 nm irradiation and a GTE-Sylarium Model CCT (energy $1{\cdot}4 \times 10^{-2}$ j/cm^2/s) for 360 nm irradiation.

Oxygenation of arachidonic acid by fetal calf skin dioxygenase

The biosynthetic pathway of PGE_2 and $PGF_{2\alpha}$ from arachidonic acid consists of several steps as shown in Fig. 1. The first and rate limiting step from the precursor arachidonic acid is the oxygenation of arachidonic acid (Smith & Lands, 1971).

Acetone powder of prostaglandin synthetase was prepared from the microsomal fraction of fetal calf skin according to the procedure described by Wallach & Daniels (1971). Oxygen concentrations were measured on a Yellow Springs Instrument Co., Model 53 oxygen monitor equipped with a model 5301 bath assembly. The monitor was attached to a Corning 800 strip recorder. The reaction mixture contained 1·5 mg of microsomal acetone powder suspended in 40 μl of 0·1 M Tris HCl buffer, pH 8·5 and 1·0 mg arachidonic acid (20:4, ω-6) dispersed in 20 μl of 0·1 M Tris buffer pH 8·5. Various concentrations of the test substances were added into the incubation chamber and sonicated before the initiation of the reaction. For incubations involving 254 nm irradiation, the UVS-54 source was placed 10 cm directly above the reaction vials which contained either the enzyme alone or enzyme plus test compound. In those experiments utilizing the 360 nm source the reaction vials were placed inside the GTE Sylarium. UV-irradiation exposure was of a 10 min duration in both cases. The oxidation of

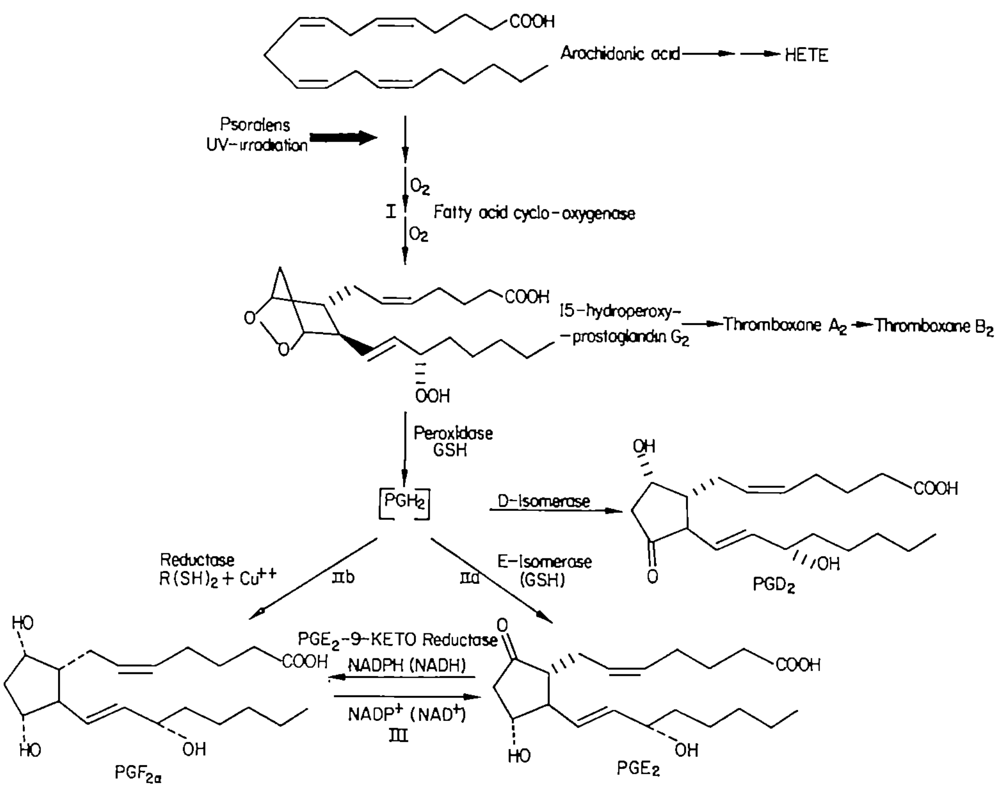


FIGURE 1. Pathways of prostaglandin biosynthesis and metabolism.
I represents the oxygenation of arachidonic acid. IIa and IIb represent the transformation of the cyclic endoperoxide into PGE_2 and $PGF_{2\alpha}$ respectively. III represents the conversion of PGE_2 into $PGF_{2\alpha}$.

arachidonic acid by the dioxygenase was determined by measuring oxygen absorption at a constant temperature of 30 ± 5°C according to the procedure of Smith & Lands (1972). The total volume of each incubation was 3·0 ml 0·1 M Tris buffer.

Biosynthesis of prostaglandins from ^{14}C-arachidonic acid

In order to determine the relative amounts of PGE_2 and $PGF_{2\alpha}$ formed from ^{14}C arachidonic acid, incubations were carried out with whole skin from normal and essential fatty acid (EFA) deficient rats and with acetone powder prepared from the microsomal fraction of fetal calf skin as sources of enzyme according to the procedure previously described in our laboratory (Ziboh *et al.*, 1971). Briefly, [1-^{14}C] arachidonic acid (2·0 μCi, specific activity 58 mCi/mM) was incubated with whole skin in 1·0 ml of 0·1 M Tris HCl buffer, pH 8·5, containing 0·55 mM hydroquinone. Various concentrations of test compounds were suspended in the medium by sonication and incubated for 1 h. In those incubations involving UV-irradiation exposure to the UV source was for the duration of the incubation period. Secondly, [1-^{14}C] arachidonic acid, 0·1 μCi, was incubated with the acetone powder preparation for 10 min (Van Dorp, 1971). Identification of radioactive products was by thin layer chromatography on silica gel G as reported previously (Ziboh, 1973).

Conversion of PGE_2 into $PGF_{2\alpha}$ by PGE_2-9-ketoreductase

The activity of PGE_2-9-ketoreductase has been localized previously in the 105,000 × *g* supernatant of a number of tissues (Lee & Levine, 1974). The enzyme preparation from skin was prepared according to the method described by Lee & Levine (1974) with the following modifications: the 105,000 × *g* supernatant fraction prepared from rat skin was partially purified and concentrated by filtering through Sephadex G-25 (coarse). Incubation procedure was as described by Lee & Levine (1974). The reaction mixture was stopped with a mixture of chloroform–methanol, 2:1 (v/v) as reported previously (Ziboh, 1973). Radioactive products were identified by thin layer chromatography on silica gel G in solvent systems: ethyl acetate–formic acid 99:1 (v/v) and chloroform–methanol–acetic acid–water, 90:8·5:1:0·7. UV-irradiation was as described in the previous section.

RESULTS

Oxygenation of arachidonic acid by fetal calf skin dioxygenase

The oxygenation of arachidonic acid is the first and rate limiting step of prostaglandin synthesis from arachidonic acid (Fig. 1, I). The effects of UV-irradiation of 254 and 360 nm with and without 8-methoxy psoralen on oxygenation reaction are shown in Fig. 2. Marked stimulation of oxygen consumption was associated with UV-irradiation at 254 nm. Although 8-methoxy psoralen without UV-irradiation exerted a stimulatory effect on oxygen consumption, this effect was enhanced 3-fold in the presence of 254 nm irradiation. Furthermore, UV-irradiation at 360 nm also enhanced the stimulatory effect of 8-methoxy psoralen on the oxygen consumption, but UV-irradiation at 254 nm was more effective in our *in vitro* system. In view of this marked effect of UV-irradiation at 254 nm on prostaglandin synthesis, subsequent experiments were carried out with a UV source at this wavelength. Under similar conditions, UV-irradiation at 254 nm enhanced the stimulatory effect of coal tar on oxygen consumption approximately 75%. Mycophenolic acid had no effect on oxygen consumption in the presence or absence of UV-irradiation.

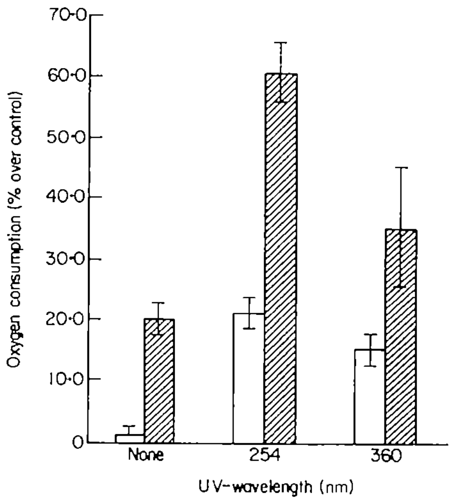


FIGURE 2. The effects of 8-methoxy psoralen (8 MOP) and UV-irradiation on the oxygenation of arachidonic acid by fetal calf skin acetone powder.

Acetone powder of microsomal fraction prepared from fetal calf skin (1·5 mg) was suspended in an assay mixture of 0·1 M Tris-HCl buffer, pH 8·5 containing 0·5 mM 8-MOP. Arachidonic acid was added to initiate the reaction under UV-light at wavelengths of 254 and 360 nm. The reaction rate (μMO_2/min = nmoles/ml per min) was determined from continuous measurements of oxygen uptake with an oxygen electrode. The data are from the mean of duplicate paired experiments. Open column, control; hatched column, +8−MOP.

Biosynthesis of prostaglandins from ^{14}C-arachidonic acid by acetone powder of fetal calf skin dioxygenase and whole rat skin

In order to ascertain whether the oxygenation of arachidonic acid was related to prostaglandin biosynthesis, the acetone powder preparation used in the above oxygen consumption assay, and ^{14}C-arachidonic acid were incubated as described under Methods. The total amount of prostaglandins biosynthesized and the relative amounts of PGE_2 and $PGF_{2\alpha}$ of each incubation were measured by thin layer chromatography as described previously (Ziboh, 1973). When tested in this assay system UV-irradiation alone at wavelengths of 254 and 360 nm both stimulated the incorporation of ^{14}C from arachidonic acid into total prostaglandins approximately 25–30% (Fig. 3a). However, in the presence of 8-methoxy psoralen (0·5 mM), the production of prostaglandins was stimulated approximately twofold at 254 nm and 20% at 360 nm (Fig. 3b). A comparative result of incubations with maximal concentrations of coal tar, mycophenolic acid and psoralens at 254 nm is shown in Fig. 4b. Psoralens with UV-irradiation at 254 nm was most effective in stimulating the conversion of arachidonic acid into prostaglandins.

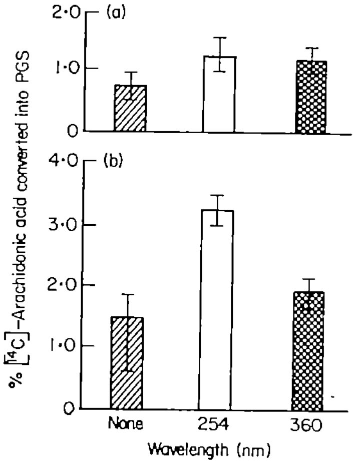


FIGURE 3. The effects of 8-MOP and UV-irradiation on the transformation of arachidonic acid into prostaglandins by fetal calf skin acetone powder.

Acetone powder of microsomal fraction from fetal calf skin (1·5 mg) was suspended in 0·1 M Tris HCl buffer, pH 8·5, containing 0·55 mM hydroquinone, 8-MOP (0·5 mM). After the addition of [1-^{14}C] arachidonic acid (0·1 μCi) the mixture was incubated at 37°C for 10 min under UV-irradiation at wavelengths of 254 and 360 nm. Identification of radioactive products was by thin layer chromatography on plates coated with silica gel G as described previously (Ziboh, 1973). The data are from the mean of duplicate paired experiments. Hatched column, control; open column +8-MOP; cross-hatched column, coal tar. (a) Control, (b)+8-MOP.

Total prostaglandins in the above experiments were separated into E and F prostaglandins by thin layer chromatography as described under Methods. The ratio of PGE_2 to $PGE_{2\alpha}$, formed from the precursor arachidonic acid as shown in Fig. 5, was depressed markedly by 8-methoxy psoralen. Furthermore, the results also demonstrated that the effect of 8-methoxy psoralen in altering the ratio is independent of the UV-irradiation. It is interesting that coal tar and mycophenolic acid had no effect on the PGE_2 to $PGF_{2\alpha}$ ratio. This result suggests that at least in part, 8-methoxy psoralen may exert an effect on prostaglandin biosynthesis by influencing the transformation of cyclic endoperoxide into $PGF_{2\alpha}$ (Fig. 1, II). The mechanism of this transformation is however unknown. Nonetheless,

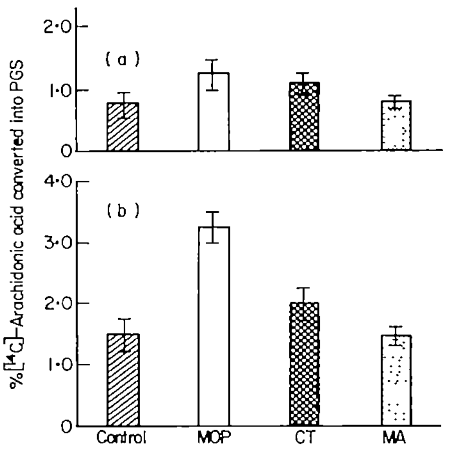


FIGURE 4. The effect of photosensitizers and UV-irradiation on the transformation of arachidonic acid into prostaglandins by fetal calf skin acetone powder.

Acetone powder of microsomal fraction from fetal calf skin (1·5 mg) was suspended in 0·1 M Tris HCl buffer, pH 8·5, containing 0·5 mM hydroquinone and 8-MOP (0·5 mM), coal tar (CT, 0·1 mM) and mycophenolic acid (MA, 0·3 mM) respectively. These concentrations were previously found to exert maximal effects on the oxygenation of arachidonic acid. After the addition of [1-^{14}C] arachidonic acid (0·1 μCi), the mixture was incubated at 37°C for 10 min. Identification of radioactive products are as indicated in Fig. 3. The data are from the mean of duplicate paired experiments. (a) No UV-light. (b) 254 nm. Hatched column, control; open column, +8-MOP (MOP); cross-hatched column, coal tar (TC); dotted column, mycophenolic acid (MA).

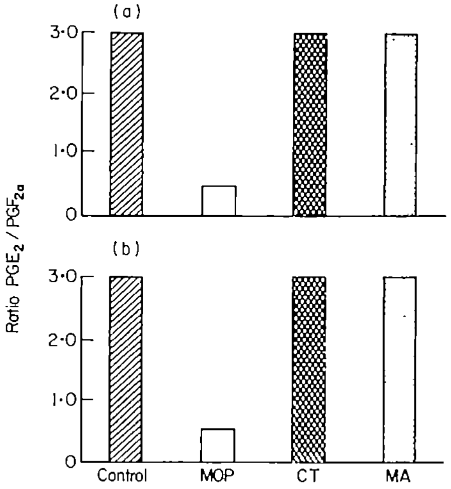


FIGURE 5. Ratio of PGE$_2$ to PGF$_{2\alpha}$ formed from the transformation of arachidonic acid into prostaglandins.

Biosynthesis of prostaglandins from [1-^{14}C] arachidonic acid by fetal calf skin acetone powder was as described in Fig. 3. Total prostaglandins were separated into E and F prostaglandins by thin layer chromatography on silica gel G plates as described previously (Ziboh, 1973). The ratio of PGE$_2$ to PGF$_{2\alpha}$ formed in the absence of UV-light and chemical agents is shown in the upper portion of figure. The lower portion represents the ratio of PGE$_2$ to PGF$_{2\alpha}$ formed in the presence of UV-irradiation and chemical agents. The data are from the mean of duplicate paired experiments. (a) No UV-light. (b) 254 nm. Hatched column, control; open column, +8-MOP (MOP); cross-hatched column, +coal tar (CT); dotted column, mycophenolic acid (MA).

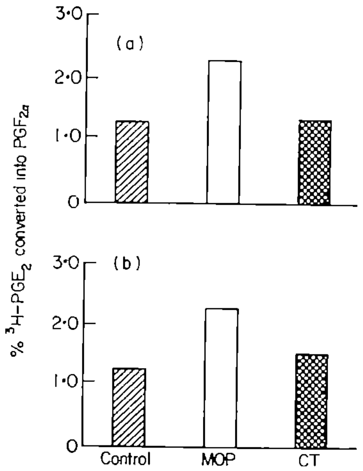


FIGURE 6. The effects of photosensitizers and UV-irradiation on the transformation of PGE_2 into $PGF_{2\alpha}$ by rat skin cytoplasmic fraction.

1 ml of partially purified 105,000 × g supernatant fraction (10 mg protein) prepared from rat skin in 0·1 M phosphate buffer, pH 7·4 containing 4 mM $MgCl_2$, 0·1 mM dithiothreitol and 10 mM NADPH was incubated with ^{3}H-PGE_2 (0·2 μCi) at 37°C for 30 min. Identification of radioactive products was by thin layer chromatography on silica gel G as described under Methods. The data are from the mean of duplicate paired experiments. (a) No UV-light. (b) 254 nm. Hatched column, control; open column, + 8-MOP (MOP); cross-hatched column, + coal tar (CT).

a number of substances have been shown to influence this transformation from cyclic endoperoxide into $PGF_{2\alpha}$ (Lands, Lee & Smith, 1971). A recent report has also suggested a non-enzymatic transformation of the cyclic endoperoxide into $PGF_{2\alpha}$ (Chan *et al.*, 1975). Further studies are necessary to clarify the formation of $PGF_{2\alpha}$ from precursor arachidonic acid.

In view of the above results, subsequent experiments with 8-methoxy psoralen were carried out at 254 nm. Incubations using whole skin from normal and EFA deficient rats were also carried out as described above with and without UV-irradiation at 254 nm. 8-Methoxy psoralen at 0·5 mM concentration again had the greatest stimulatory effect on the biosynthetic enzyme system.

Conversion of PGE_2 to $PGF_{2\alpha}$ by PGE_2-9-ketoreductase preparations from normal and EFA deficient rats

Since the preceding experiment showed a marked alteration of the PGE_2 to $PGF_{2\alpha}$ ratio by psoralen from precursor arachidonic acid, we next investigated whether or not 8-methoxy psoralen and the other substances exert any effects on the activity of the PGE_2-9-ketoreductase, an enzyme known to catalyse the transformation of PGE_2 into $PGF_{2\alpha}$ as indicated in Fig. 1, III. The effects of 8-methoxy psoralen, coal tar and UV-irradiation at 254 nm on the transformation of PGE_2 into $PGF_{2\alpha}$ by enzyme preparations from normal rat skin are shown in Fig. 6b. 8-Methoxy psoralen stimulated the conversion of PGE_2 into $PGF_{2\alpha}$ approximately two-fold, whereas coal tar had no effect on this enzymatic transformation. Similar results were obtained with enzyme preparations from skin of EFA deficient rats. The results also showed that the effect of PGE_2 transformation is independent of the UV-irradiation.

DISCUSSION

This study has demonstrated that exposure of skin preparations *in vitro* to UV-irradiation wavelengths of 254 nm and 360 nm results: (i) in the stimulation of the oxygenation of arachidonic acid (Fig. 2);

(ii) in the incorporation of ^{14}C from arachidonic acid into prostaglandins (Fig. 3a). These *in vitro* results therefore support previously reported increases in prostaglandin levels in the skin after UV-irradiation (Greaves *et al.*, 1970; Mathur *et al.*, 1972; Synder *et al.*, 1974). Although the mechanism of action of UV-induced biosynthesis of prostaglandins cannot be delineated from the present studies, it is nonetheless likely that UV-irradiation is involved early in the initiation of a free-radical fatty acid intermediate from arachidonic acid which then serves as a reactive substrate for the attack of O_2. This free-radical–oxygen complex is then transformed by the dioxygenase enzyme into hydroperoxide and cyclic endoperoxide intermediates. Our results also demonstrate that UV-irradiation is not involved in the metabolic transformations of E and F prostaglandins, thus suggesting further that its site of action may be involved early in the formation of free-radicals involved in prostaglandin biosynthesis. A mechanism similar to the above has been reported to occur when cells are exposed to light thus resulting in the formation of a cyclic peroxidation of tri-unsaturated fatty acid (Heath & Packer, 1968).

Our results also demonstrate that exposure of skin preparations to 8-methoxy psoralen and UV at 254 nm result in a marked stimulation of prostaglandin biosynthesis (Fig. 3b). The stimulatory effect of 8-methoxy psoralen was lower with UV at 360 nm. In similar experiments, coal tar and UV-irradiation had a moderate stimulatory effect on the incorporation of ^{14}C from arachidonic acid into prostaglandins whereas mycophenolic acid had no effect (Fig. 4). The mechanism of the stimulatory effect of 8-methoxy psoralen and coal tar in the presence of UV-irradiation on prostaglandin biosynthesis is unknown. It is interesting however that photosensitizers in the presence of light have been reported to play a role in lipid peroxidation (Obrien, 1969; Heath & Packer, 1968). Further studies are in progress to elucidate this mechanism of action.

It was also interesting to note that incubations of precursor arachidonic acid with skin microsomal preparations and 8-methoxy psoralen resulted in a marked decrease of the $PGE_2/PGF_{2\alpha}$ ratio (Fig. 5). This result would suggest that the alteration was due to the increased formation of $PGF_{2\alpha}$ from precursor fatty acid and not from the metabolism of PGE_2 to $PGF_{2\alpha}$ catalysed by a soluble 9-keto-PGE2-reductase. Whether the effect of 8-methoxy psoralen in preferentially stimulating the formation of $PGF_{2\alpha}$ from arachidonic is on the cofactors or on the enzyme(s) in the biosynthetic pathway is unknown and deserves further study. However, several compounds have been reported to enhance the transformation of cyclic endoperoxide into $PGF_{2\alpha}$ (Lands *et al.*, 1971). Furthermore, a recent report has suggested the possible non-enzymatic transformation of the cyclic endoperoxide into $PGF_{2\alpha}$ catalysed by ions and some compounds (Chan *et al.*, 1975).

Of particular interest in this study is the observation that 8-methoxy psoralen also stimulated the transformation of PGE_2 into $PGF_{2\alpha}$ (Fig. 6). This effect is independent of light, suggesting a possible effect of 8-methoxy psoralen directly on the PGE_2-9-ketoreductase, or on the cofactors necessary for this enzymatic transformation. Our present data are however inadequate to delineate whether or not the effect of this photosensitizer is on the NADPH cofactor or directly on the enzyme PGE_2-9-ketoreductase. Further studies are in progress to elucidate this mechanism of action of the psoralens and compounds of similar properties on the metabolism of prostaglandins.

Since the E-and F-prostaglandins qualitatively exert different pharmacological effects on several tissues (Bergstrom, Carlson & Weeks, 1968; Horton, 1965), substances capable of altering their concentrations in the tissue could conceivably exert important physiological effects on the tissues. Our goal, in the pursuit of these studies, was to ascertain whether or not the therapeutic use of the psoralens, coal tar and mycophenolic acid with UV-irradiation in the treatment of psoriasis has any relationship to the biosynthesis and metabolism of prostaglandins in the skin. It is reasonable to speculate on such a possibility since alterations in prostaglandin levels have been reported in non-involved and involved skin of psoriatic patients (Aso *et al.*, 1975; Hammerstrom *et al.*, 1975). Thus, compounds capable of altering the levels of E-and-F prostaglandins may play a role in the control of the pathophysiological processes in this disease process. A further possible role of prostaglandin in psoriasis was indicated by Penneys *et al.*, (1975, 1976) who reported that skin homogenates prepared from a psoriatic plaque and from the white rim (Woronoff's Ring) that surrounds the plaque inhibit the transformation of arachidonic acid into prostaglandins. A similar result was observed by Ziboh & Lord with skin homogenates prepared from EFA-deficient rats (unpublished report). Additionally, Ziboh *et al.* (1975) reported an increase in the conversion of PGE_2 into $PGF_{2\alpha}$ by skin specimens from psoriatic plaque and from EFA deficient rats. The above reports suggest that the abnormality in the biosynthesis and metabolism of prostaglandin may be related, at least in part, to the pathophysiology of psoriasis.

The mechanism of action of 8-methoxy psoralen and coal tar in conjunction with UV in the management of psoriasis is unclear. Nonetheless these substances under UV-irradiation exert marked effects on the biosynthesis and metabolism of prostaglandins in the skin. Since prostaglandins play important biochemical and physiological functions in the skin, it is reasonable to speculate that the effects of the photosensitizers on the skin may, at least in part, be via their regulation of prostaglandin biosynthesis and metabolism in the skin.

ACKNOWLEDGMENTS

This investigation was supported by Research Grant AM-14941 of the U.S. Public Health Service and Research Grant from The National Psoriasis Foundation.

The authors express their thanks to Ms Ileana Ruiz for typing the manuscript and preparation of the figures.

REFERENCES

Aso, K., Deneau, D.G., Krulig, L., Wilkinson, D.I. & Farber, E.M. (1975) Epidermal synthesis of prostaglandins and their effects of levels of cyclic adenosine 3'-5' monophosphate. *Journal of Investigative Dermatology*, **64** 326.

Berstrom, S., Carlson, L.A. & Weeks, J.R. (1968) The prostaglandins: a family of biological active lipids. *Pharmacological Review*, **20**, 485.

Chan, J.A., Nagasawa, M., Takeguchi, C., & Sih, C.J. (1975) On agents favoring prostaglandin F formation during biosynthesis. *Biochemistry*, **14**, 2987.

Fitzpatrick, T.B., Hopkins, E.C., Blickenstaff, D.D. & Swift, S. (1955) Augumented pigmentation and other responses of normal skin to solar pigmentation following oral administration of 8-methoxy psoralens. *Journal of Investigative Dermatology*, **25**, 187.

Goeckerman, W.H. (1931) Treatment of psoriasis. *Archives of Dermatology*, **24**, 446.

Greaves, M.W. & Sondergaard (1970) Pharmacologic agents released in ultraviolet inflammation studied by continuous skin perfusion. *Journal of Investigative Dermatology*, **54**, 365.

Greaves, M.W. & McDonald, W. (1972) Prostaglandin biosynthesis by human skin and inhibition by corticosteroids. *British Journal of Pharmacology*, **46**, 172.

Hammerstrom, S., Hamberg, M., Samuelsson, B., Duell, E.A. Stawiski, M. & Voorhees, J.J. (1975) Elevated levels of free arachidonic acid, PGE_2, $PGF_{2\alpha}$ and 12L-Hydroxy-5,8,10,14-eicosatrienoic acid (HETE) in rapidly proliferating, incompletely differentiated epidermis of psoriasis. Abstracts of papers. *International Conference on Prostaglandins*, Florence, Italy.

Heath, R.L. & Packer, L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and Stoichiometry of fatty acid peroxidation. *Archives of Biochemistry & Biophysics*, **125**, 189.

Heath, R.L. & Packer, L. (1968) Photoperoxidation in isolated chloroplasts. II. Role of electron transfer. *Archives of Biochemistry & Biophysics*, **125**, 850.

Horton, E.W. (1965) Biological activities of pure prostaglandins. *Experientia*, **XXI**, 113.

Jonsson, C.D. & Angaard, E. (1972) Biosynthesis and metabolism of prostaglandin E_2 in human skin. *Scandinavian Journal of Clinical Laboratory Investigation*, **29**, 289.

LANDS, W., LEE, R. & SMITH, W. (1971) Factors regulating the biosynthesis of various prostaglandins. *Annals of New York Academy of Science*, **181**, 102.

LEE, S., & LEVINE, L. (1974) Prostaglandin metabolism: Cytoplasmic reduced nicotinamide adenine dinucleotide phosphate-dependent and microsomal reduced nicotinamide adenine dinucleotide dependent prostaglandin E-9 ketoreductase activities in monkey and pigeon tissues. *Journal of Biological Chemistry*, **249**, 1369.

MATHUR, G.P. & GANDHI, V.M. (1972) Prostaglandin in human and albino rat skin. *Journal of Investigative Dermatology*, **58**, 291.

OBRIEN, P.J. (1969) Intracellular mechanisms for the decomposition of a lipid peroxide. I. Decomposition of a lipid peroxide by metal ions, heme compounds and nucleophiles. *Canadian Journal of Biochemistry*, **47**, 485.

PATHAK, M.A. (1961) Mechanism of psoralen photosensitization *in vivo*: Biological action spectrum of 8-methoxy psoralen. *Journal of Investigative Dermatology*, **37**, 397.

PENNEYS, N.S., ZIBOH, V.A., LORD, J.T. & SIMON, P. (1975) An inhibitor of prostaglandin synthesis in psoriatic plaque. *Nature*, **254**, 351.

PENNEYS, N.S., ZIBOH, V.A., SIMON, P. & LORD, J. (1976) Pathogenesis of a Woronoff's Ring. *Archives of Dermatology*, in press.

SMITH, W.L. & LANDS, W.E. (1971) Stimulation and blockade of prostaglandin biosynthesis. *Journal of Biological Chemistry*, **246**, 6700.

SMITH, W.L. & LANDS, W.E. (1972) Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland. *Biochemistry*, **11**, 2376.

SNYDER, D.S. & EAGLSTEIN, W. (1974) Intradermal anti-prostaglandin agents and sunburn. *Journal of Investigative Dermatology*, **62**, 47.

SONDERGAARD, J., PEKKA, J. & JORGENSEN, H.O. (1973) Human cutaneous inflammation induced by prostaglandin E_1. *Journal of Pathology*, **109**, 239.

WALLACH, D.O. & DANIELS, E.G. (1971) Properties of a novel preparation of prostaglandin synthetase from sheep seminal vesicles. *Biochemica et Biophysica Acta*, **231**, 445.

VAN DORP, D. (1971) Recent developments in the biosynthesis and the analysis of prostaglandins. *Annals of New York Academy of Science*, **180**, 181.

ZIBOH, V.A. & HSIA, S.L. (1971) Prostaglandin E_2: Biosynthesis and effects on glucose metabolism in rat skin. *Archives of Biochemistry & Biophysics*, **146**, 100.

ZIBOH, V.A. (1973) Biosynthesis of prostaglandin E_2 in human skin: subcellular localization and inhibition by unsaturated fatty acids and anti-inflammatory drugs. *Journal of Lipid Research*, **14**, 337.

ZIBOH, V.A., LORD, J.T. & PENNEYS, N.S. (1975) Metabolism of PGE_2 by human and rat skin. Abstracts of Papers. *International Conference on Prostaglandins*, Florence, Italy.

British Journal of Dermatology (1976) **95**, 407.

Subcutaneous fat necrosis of the newborn

LIGHT AND ELECTRON MICROSCOPIC STUDIES

TAKUO TSUJI

Department of Dermatology, Osaka City University Medical School, Abeno, Osaka 545, Japan

Accepted for publication 30 January 1976

SUMMARY

Specimens from two cases of subcutaneous fat necrosis of the newborn were studied by light and electron microscopy. Crystallization of the fat cell started with clearing of the central fat droplet, followed by the appearance of cylindrical crystals. The fat crystals were phagocytosed by cytoplasmic projections of macrophages as well as by foreign body giant cells. Calcium deposits were demonstrated within the central fat droplet of the degenerated fat cell of Case 2.

Subcutaneous fat necrosis of the newborn, though there is much terminological confusion, has combined clinical and histological features that permit its ready diagnosis (Montgomery, 1967). The disease is usually seen in apparently healthy full-term infants and is characterized by nodular thickening of the subcutaneous tissues. In the areas of induration the subcutaneous fat shows patchy necrosis, varying in degree, with a granulomatous infiltration consisting of lymphocytes, epithelioid cells and foreign-body giant cells, many of which contain needle-shaped fat crystals (Lever, 1967; Montgomery, 1967). In older lesions, calcification may be present (Martin & Steven, 1957; Wesener, 1957; Duhn, Schoen & Siu, 1968). Ultrastructural features of the disease have not to our knowledge been previously presented in the literature.

The purpose of this paper is to describe the light and electron microscopical findings of biopsy specimens from two patients with this disease.

CASE REPORTS

Case 1

A Japanese male infant was born in a state of asphyxia by Caesarean section, with a birth weight of 3·8 kg. Immediate resuscitative measures were taken and subsequent improvement continued with parenteral therapy. At birth there were hard reddened discrete areas on the left buttock and both thighs. The next day, similar infiltrations appeared on the left forearm and right leg, while the infiltrations on the left buttock had spread upward to the lumbar region. Subsequently, however, no other new infiltration had appeared. On the 24th day, the infant was referred to our clinic. Examination revealed hard subcutaneous infiltrations ranging from the size of a pigeon's egg to that of a hen's egg. Biopsy specimens were obtained from the left thigh for both light and electron microscopy.

Case 2

A Japanese female infant was delivered vaginally after prolonged labour with a birth weight of 3·3 kg. The membranes had ruptured early in labour. A firm, nontender area of induration was observed on the back and shoulder. By the age of 3 weeks, areas of induration had spread to the chest, both axillary regions, and the lumbar region. On about the 25th day, the patient had a draining, purulent abscess in the centre of the area of induration on the back. On the 32nd day, the patient was referred to our clinic. Examination showed reddened, firm skin over the back, shoulder, chest, both axillary regions and lumbar region, and an abscess the size of a hen's egg in the centre of the back. Biopsy specimens were obtained from the lumbar area and chest for both light and electron microscopy.

MATERIALS AND METHODS

For light microscopy, half of each specimen was used for preparation of routine sections and the other half was for that of frozen sections. Routine 5 μm sections were cut and stained with Haematoxylin-Eosin or Von-Kossa. Frozen sections were observed with a polarization microscope and some of them were stained with Sudan III. For electron microscopy, biopsy specimens were pre-fixed at 4°C for 1 h with 2% glutaraldehyde buffered with 0·2 M phosphate (pH 7·4). After thorough rinsing they were immersed in 1% osmium tetroxide buffered with 0·2 M phosphate (pH 7·4) for 1 h and then dehydrated by graded ethyl alcohol and embedded in epon 812 according to the method of Luft

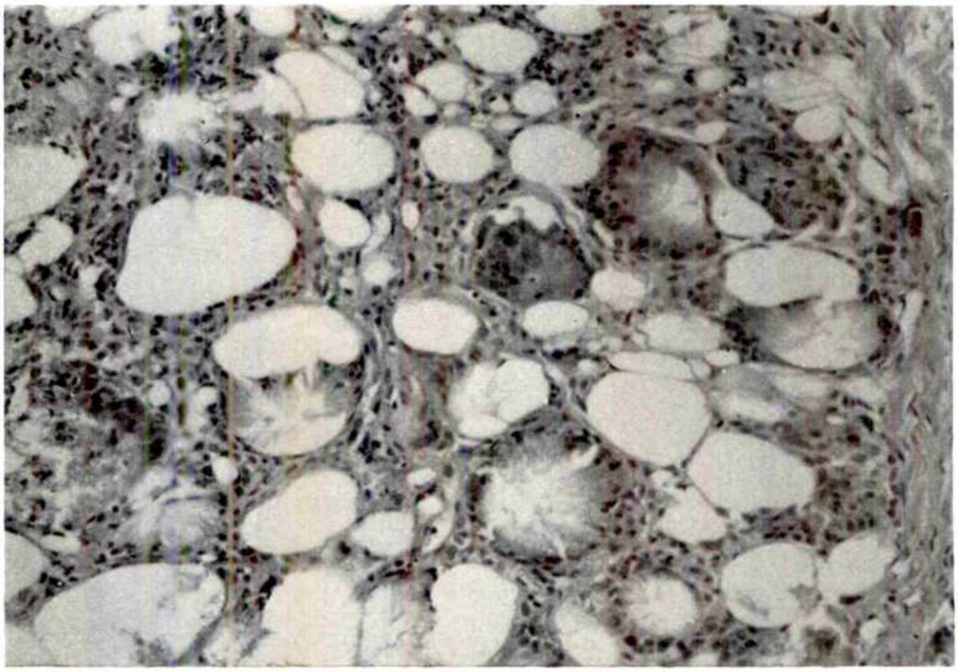


FIGURE 1. Needle-shaped clefts or spaces in the fat cells, areas of degeneration and necrosis, and a foreign-body inflammatory reaction can be seen in the subcutaneous tissue (Case 1, H & E, × 200).

(1961). Specimens were cut with a Porter-Blum MT-2 ultramicrotome. Thin 1 μm sections were stained with 1% toluidine blue and observed with the light microscope. Ultra-thin sections were stained on the grid for 1 h with a saturated aqueous solution of uranyl acetate followed by immersion for 10 min in Reynold's lead citrate and examined with a Hitachi HS9 electron microscope.

RESULTS

Light microscopy

Degeneration, necrosis and crystallization of the subcutaneous fat, together with an inflammatory reaction and fibrosis, were seen (Fig. 1). The fat cells of the subcutis varied greatly in size and in shape. Fat in the fat cells was dissolved during the preparation of a paraffin section and therefore could not be stained with H. & E. Needle-shaped empty clefts lay singly or in radial arrangement inside fat cells and in the foreign-body giant cells. They failed to stain with fat stains, but were doubly refractile in the polarization microscope. In Case 2, calcium deposits were demonstrated within the fat cell by Von-Kossa stain. In the thin sections of epon-embedded tissues stained with toluidine

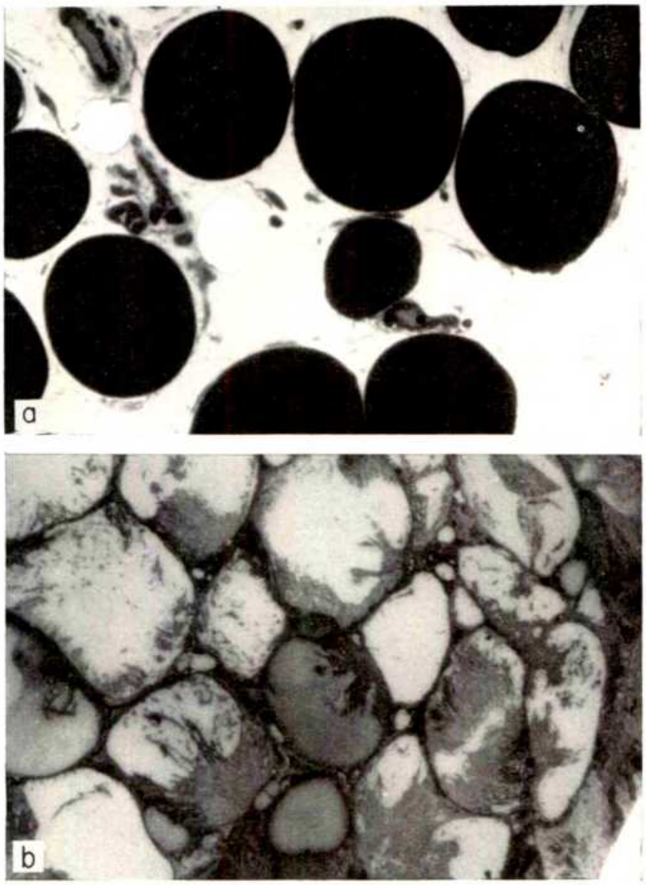


FIGURE 2. (a) The central fat droplets of normal fat cells show uniformly dark blue (a toluidine blue-stained epoxy section, × 200). (b) Amorphous projections protruding in various degrees from the peripheral portion of the fat cell into the fat droplet are seen (a toluidine blue-stained epoxy section, × 200).

blue, the fat droplets of normal fat cells showed up uniformly dark blue (Fig. 2a); fat droplets of abnormal cells were stained irregularly dark to pale blue, some of them showing an amorphous material protruding from the periphery of the cell into the fat droplet (Fig. 2b). By increasing the magnification, empty, needle-shaped clefts were seen within the amorphous material. Within the foreign-body giant cells the needle-shaped clefts occasionally lay in a radial arrangement. The differences of the lesions between Cases 1 and 2 were of a quantitative rather than a qualitative character, except for the deposition of calcium in Case 2. In Case 1 there were fat cells in various degrees of degeneration, and only a few foreign-body giant cells. In Case 2, on the other hand, there were fewer fat cells and more foreign-body giant cells.

Electron microscopy

Normal fat cells of the neonatal adipose tissue are similar to those of the adult except for their size. The cytoplasm formed a very thin rim around the large central fat droplet, except in the region of the nucleus where it was somewhat thicker. The cytoplasm contained the common organelles, and the interface between cytoplasm and central fat droplet was smooth. The central fat droplet was observed as a moderately regular electron-dense material, with no empty spaces.

Degenerated fat cells. Fat cells in various degrees of degeneration, from relatively healthy cells to degenerated necrotic ones, were seen. In relatively healthy cells, changes were observed only in the central fat droplet. The fat droplet consisted of two components; a moderately electron-dense

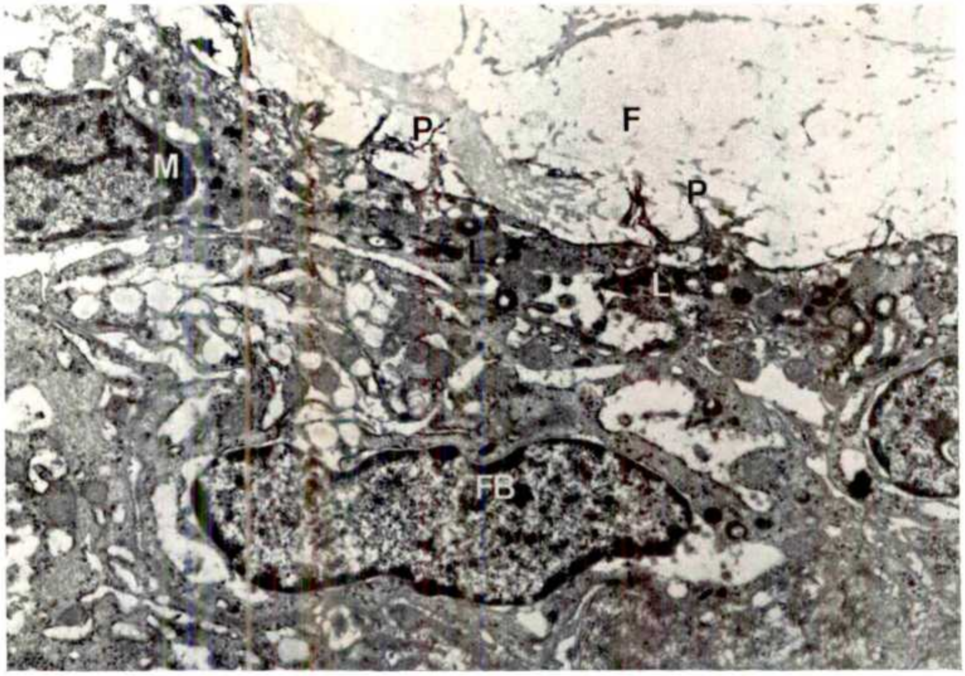


FIGURE 3. Irregular strand-like projections (P) are seen around the central fat droplet (F). These projections are continuous with a macrophage (M) which contains many lysosomal structures (L). FB, fibroblast (×8000).

amorphous component and an electron translucent component. These showed an irregular reticular or vermiform appearance with both intertwined with each other. On the other hand, there were no visible changes in the cytoplasm. The interface between cytoplasm and central fat droplet remained smooth. In rather more degenerated cells there were various degrees of invasion of projections from the peripheral portion of the cell into the central fat droplet (Figs 3, 4a). The projections were mainly composed of amorphous or fine reticular substances and contained numerous empty spaces of irregular, spindle-like or cylindrical form. At the margins of the projections, there were thin membranous folds which were adherent to adjacent folds so as to entrap part of the fat droplet in a form of phagocytosis (Fig. 4b). Cellular organelles were rare in this area. At the root of the projections many lysosomal structures as well as empty spaces were seen (Fig. 4a). The rim of fat cell cytoplasm was indistinct and parts of the projections appeared to be in direct contact with the connective tissue septum. Examination of serial sections revealed that these projections were derived from macrophages which were present in the connective tissue septum between degenerated fat cells. Figs 3 and 4a show that the projections are continuous with a macrophage the cytoplasm of which contains many lysosomal structures. With more advanced degeneration, fat cells were occupied with the projections of macrophages and some of them showed the appearance of a foreign-body giant cell.

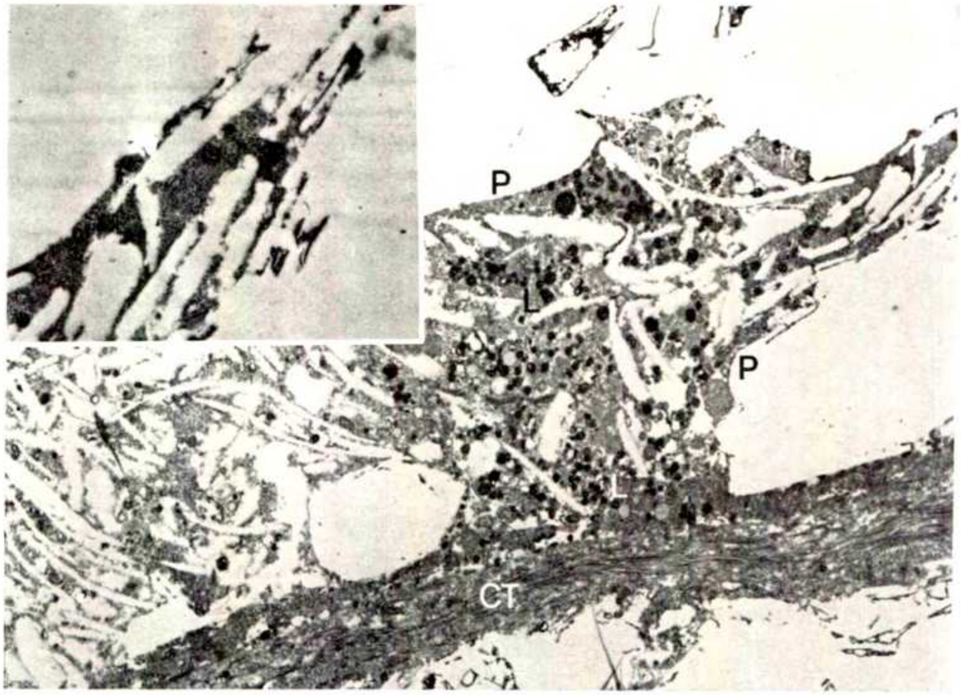


FIGURE 4. Projections (P) containing numerous empty spaces of irregular or cylindrical form are seen. At the root of the projections, many lysosomal structures (L) can be seen. CT, connective tissue septum (× 5000).
Inset. At the margins of the projections, there are thin membraneous folds which are adherent to adjacent folds so as to entrap part of the fat droplet in a form of phagocytosis (× 10,000).

Foreign-body giant cells. Varying numbers of apparently empty spaces, cylindrical, needle-like,

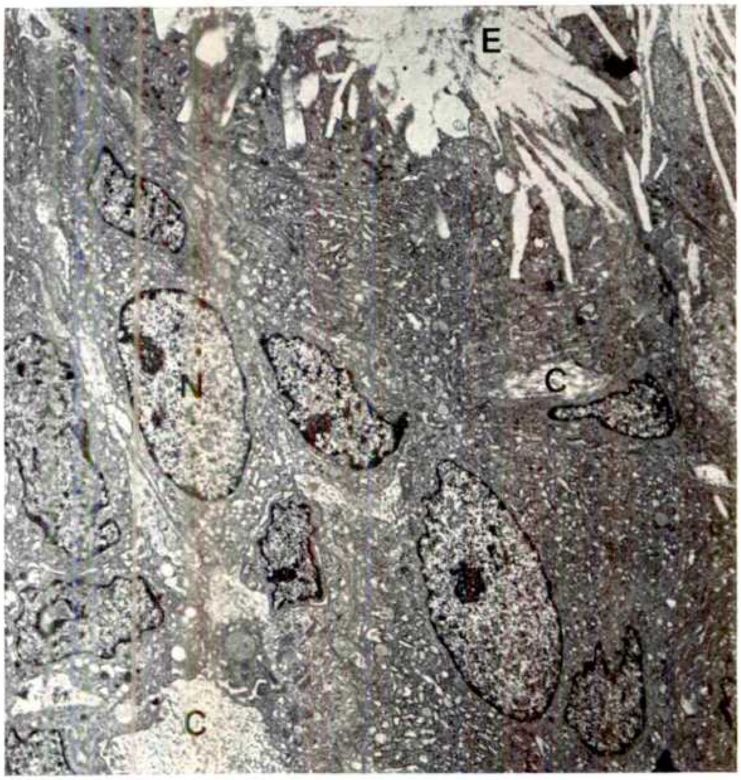


FIGURE 5. Foreign-body giant cell. Empty spaces (E) are seen radiating from the centre to the peripheral portion of the giant cell. Three or four cell layers can be seen around the empty spaces area. C, collagen fibers; N, nucleus (× 3000).

round, oval, or irregular in shape, were seen radiating from the centre of the giant cell (Fig. 5). Some of these spaces were in clusters towards the centre where they were often free of any visible limiting membrane and were separated from each other by only a thin septum of the cytoplasmic remnant (Fig. 6). Some empty spaces appeared to be retained among the many deep, narrow cytoplasmic invaginations of the giant cell. Other spaces were scattered in peripheral portions of the cell, most of them being bound by a limiting membrane. These empty spaces were lying within the cytoplasm of the cell and many lysosomal structures were seen around the empty spaces (Fig. 6). The more peripheral portion of the giant cell contained numerous mitochondria, extensive Golgi complexes, vesicles, lysosomal structures and fine filaments as well as empty spaces of various shapes. The outermost region of the cell contained abundant rough-surfaced endoplasmic reticula arranged in cisternae or closed apposed lamellae (Fig. 7). Groups of lipid droplets were also seen. The cellular outline of the giant cell was irregular with many projecting wavy pseudopodia. The cells forming the giant cell were attached to each other by interlocking pseudopodia. Sometimes degenerated fat cells were enclosed by these pseudopodia or were contained within the giant cell.

Calcium deposition. Clusters of highly electron-dense granules were seen in the moderately electron-dense component of the central fat droplet (Fig. 8). In some fat cells, the granules were found along the margin of the fat droplet, while in others they were distributed over almost all of the droplet. Most

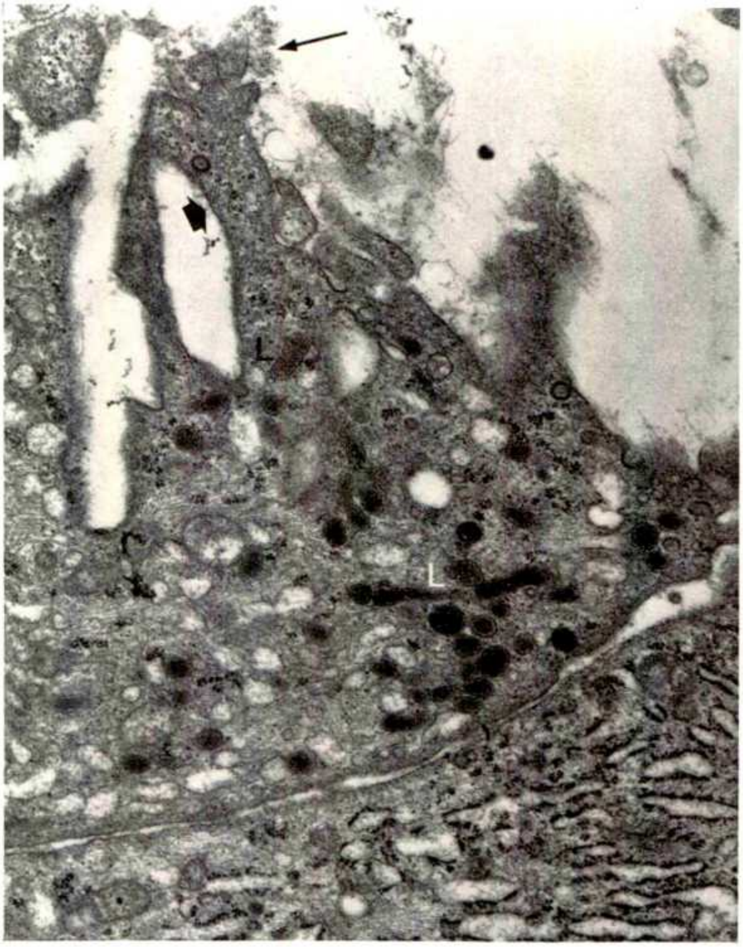


FIGURE 6. The centre of the giant cell (higher magnification of Fig. 5). Empty spaces are in a cluster; some of these are free of any visible limiting membrane (thin arrow), and others are bound by a limiting membrane (thick arrow). Many lysosomal structures (L) are seen around the empty spaces (× 13,000).

of the granules were very small and formed round aggregates, but a few of them formed clusters of filamentous or needle-like structures resembling thin crystals. These granules seemed identical to the material stained black with Von-Kossa stain, i.e. calcium salts. They were present not only in the fat droplets of degenerated fat cells, but also in the fat droplets contained within giant cells. However, there were no granules within the empty spaces of either the fat cell or the giant cell.

DISCUSSION

Crystallization in fat cells

The present study confirmed that there were various degrees of degeneration of fat cells, ranging from relatively healthy to necrotic cells, some of which were phagocytosed by giant cells. One of the impor-

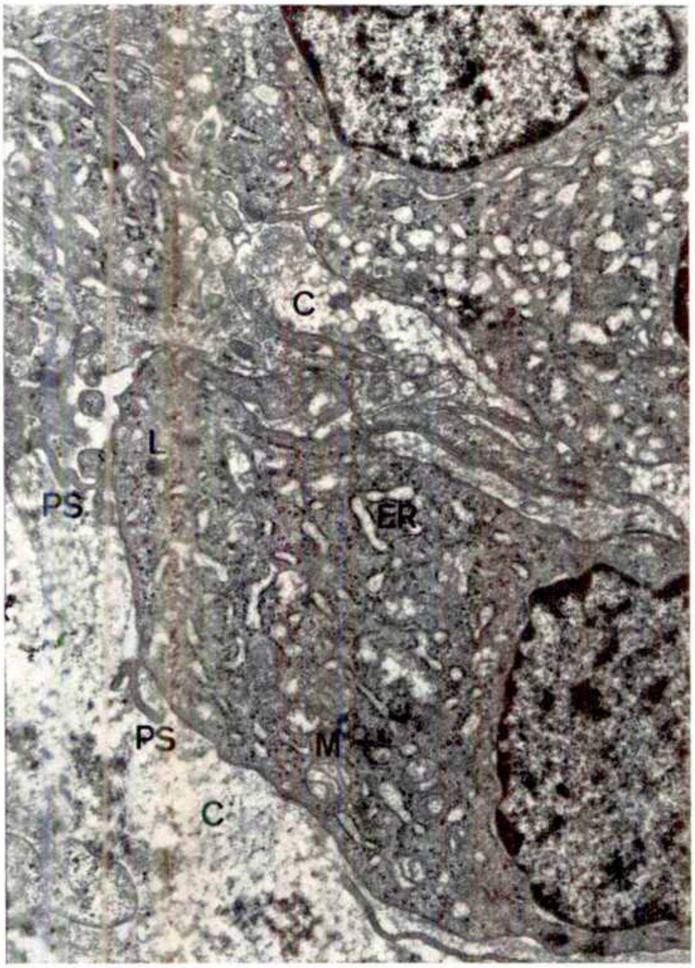


FIGURE 7. The peripheral portion of the giant cell (higher magnification of Fig. 5). Mitochondria (M), lysosomal structures (L) and rough-surfaced endoplasmic reticular (ER) are seen. C, collagen fibres (× 10,000).

tant and remarkable changes was crystallization of the central fat droplet inside the fat cells. On the basis of observation of these degenerated fat cells, it is surmised that the crystallization starts with partial clarification of the fat droplet, and is completed by the formation of cylindrical or needle-shaped crystals. A reticular or vermiform appearance probably indicates a transitional stage of crystallization. Since the fat crystals themselves are not visible, they can be recognized only when enclosed by visible structures. Under the electron microscope fat crystals could be seen within cytoplasmic projections from macrophages invading the fat cell and within the cytoplasm of foreign-body giant cells. The chemical nature of the fat crystal is uncertain. It may be cholesterol and its esters (Carol & van der Zande, 1926), neutral fat (Noojin, Pace & Davis, 1949), fatty acids (Horsfield & Yardley, 1965; Lindlar & Misgeld, 1967), etc. However, it is difficult to decide from the ultrastructural character which of these is correct.

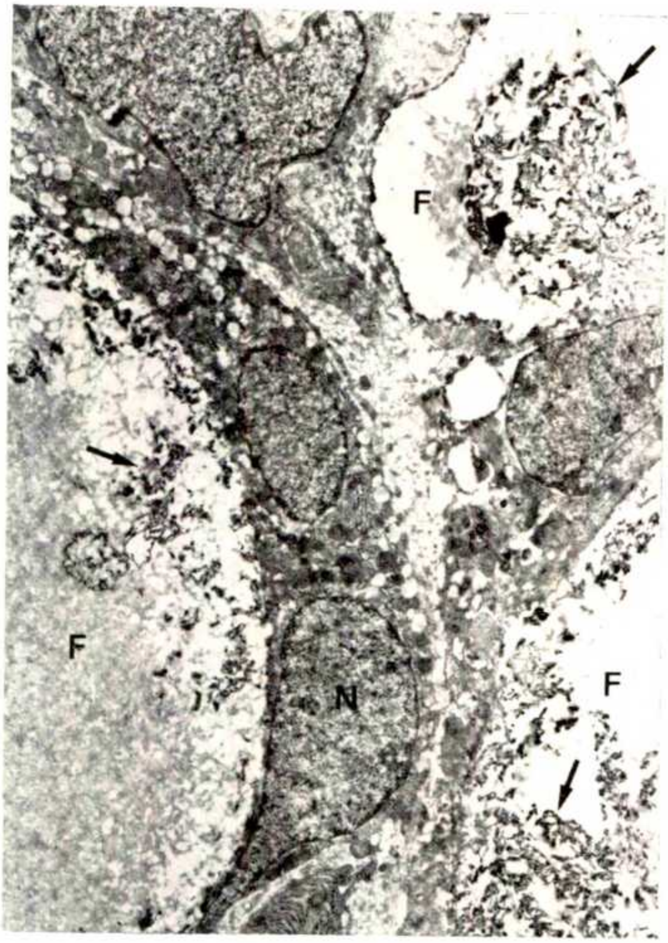


FIGURE 8. Calcium deposits within degenerated fat cells. Clusters of electron-dense granules (arrows) are seen in the moderately electron-dense component of the central fat droplet (F) (× 6000).

The phagocytosis of fat crystals

Present work demonstrated that fat crystals were phagocytosed by macrophages in relatively new lesions, while they were phagocytosed by foreign-body giant cells in older lesions. The mode of phagocytosis by macrophages was similar to that by foreign-body giant cells, except that the former contained only part of the fat cell, but the latter contained the complete cell. This suggests that there is close relationship between macrophages and foreign-body giant cells. In fact, some reports have indicated that foreign-body giant cells are formed by fusion of macrophages (Gillman & Wright, 1966; Sutton, 1967; Carter & Roberts, 1971; Mariano & Spector, 1974). It is surmised from these reports that the phagocytosis of fat crystals starts with the invasion of cytoplasmic projections of macrophages and is completed in the formation of foreign-body giant cell which results from the fusion of these macrophages.

Calcium deposits

Another interesting finding was the calcium deposits seen within the degenerated fat cells in Case 2.

Several workers (Martin & Steven, 1957; Wesener, 1957; Duhn *et al.*, 1968) have reported the demonstration by light microscopy of calcium deposits in adipose tissue in this disease, though there have been no reports of electron microscope studies. The present study showed that calcium deposition occurred only in the abnormal central fat droplets, except for the empty areas occupied by fat crystals, and originated in the peripheral portion of the fat droplet and extended to the centre. The mechanism of fat cell calcification in our patients is poorly understood. However, it is assumed that calcium deposition results from fat cell damage. According to Wheeler *et al.* (1952), damaged tissue may develop a lowered carbon dioxide tension, so that fewer calcium and phosphate ions can be held in solution. Moreover, according to Kendrick, Phalen & Hartman (1966) fat cell necrosis liberates free fatty acids which may combine with calcium ions producing calcium soaps.

REFERENCES

CAROL, W.L.L. & VAN DER ZANDE, F. (1926) Adiponecrosis subcutanea neonatorum. *Acta dermato-venereologica*, **7**, 180.

CARTER, R.L. & ROBERTS, J.D.B. (1971) Macrophage and multinucleate giant cells in nitrosoquinolone-induced granulomata in rats: an autoradiographic study. *Journal of Pathology*, **105**, 285.

DUHN, R., SCHOEN, E. J. & SIU, M. (1968) Subcutaneous fat necrosis with extensive calcification after hypothermia in two newborn infants. *Pediatrics*, **41**, 661.

GILLMAN, T. & WRIGHT, L.J. (1966) Probable *in vivo* origin of multi-nucleated giant cells from circulating mononuclears. *Nature*, **209**, 263.

HORSFIELD, G.I. & YARDLEY, H.J. (1965) Sclerema neonatorum. *Journal of Investigative Dermatology*, **44**, 326.

KENDRICK, J.I., PHALEN, G.S. & HARTMAN, J.T. (1966) Calcifications in soft tissue. Clinical significance. Conditions to consider. *Postgraduate Medicine*, **39**, 165.

LEVER, W.F. (1967) Sclerema neonatorum. In: *Histopathology of the skin*, 4th edn, p.250. J.B. Lippincott Company, Philadelphia.

LINDLAR, F. & MISGELD, V. (1967) Die Adiponecrosis subcutanea neonatorum unter lipoidchemischen Aspekt. *Hautarzt*, **18**, 115.

LUFT, J.H. (1961) Improvement in epoxy resin embedding methods. *Journal of Biophysical and Biochemical Cytology*, **9**, 409.

MARIANO, M. & SPECTOR, W.G. (1974) The formation and properties of macrophage polykaryons (inflammatory giant cells). *Journal of Pathology*, **113**, 1.

MARTIN, M.M. & STEVEN, E. M. (1957) Subcutaneous fat necrosis of the newborn with calcification of the tissues. *Archives of Disease in Childhood*, **32**, 146.

MONTGOMERY, H. (1967) Panniculitis. In: *Dermatopathology*, p.705. Harper & Row, New York.

NOOJIN, R.O., PACE, B.F. & DAVIS, H.G. (1949) Subcutaneous fat necrosis of the newborn: certain etiologic consideration. *Journal of Investigative Dermatology*, **12**, 331.

SUTTON, J.S. (1967) Ultrastructural aspects of *in vitro* development of monocytes into macrophages, epithelioid cells and multinucleated giant cells. *National Cancer Institute Monograph*, **26**, 71.

WESENER, G. (1957) Zur Klinik und Therapie der Adiponecrosis subcutanea neonatorum. *Archiv für Klinische und Experimentelle Dermatologie*, **206**, 531.

WHEELER, C.E., CURTIS, A.C., CAWLEY, E.P., GREKIN, R.H. & ZHEUTLIN, B. (1952) Soft tissue calcification, with special reference to its occurrence in the collagen diseases. *Annals of Internal Medicine*, **36**, 1050.

British Journal of Dermatology (1976) **95**, 417.

Metal corrosion by chloride in sweat

THE PROBLEM OF 'RUSTERS' IN INDUSTRY

J.L.BURTON, R.J.PYE AND D.B.BROOKES*

Department of Dermatology, Bristol Royal Infirmary, Bristol BS2 8HW and
* Beaumont Hospital, Lancaster LA1 2JF

Accepted for publication 17 January 1976

SUMMARY

Workers in the precision engineering industry who readily corrode metal are known as 'rusters'. We have described two patients with this condition, and review current ideas with regard to its cause, detection, prevention and treatment.

The ability of sweat to corrode metal has been recognized for many years (Huff, 1921). Experienced workers in the precision engineering industry also know that a few people secrete sweat which corrodes metal to a much greater extent than usual. Polished metal surfaces touched by these individuals will develop rusty fingerprints within a few days, and in addition to causing an unsightly blemish on the metal, the rust is likely to cause malfunction of complex small components such as the tumblers of locks, and equipment used in the electronics industry. People with this idiosyncracy are known in the trade as 'rusters' and the workers often incorrectly attribute the condition to excessive 'acidity' of the sweat. 'Rusters' often are unable to wear gloves to protect the metal because of the high degree of manual dexterity which their jobs require, and such employees, once detected, are therefore likely to lose their jobs.

The condition is not rare, but it does not appear to have been described previously in the dermatology literature, and it is not mentioned in standard reference texts of Dermatology or Occupational Medicine. Investigations of the problem of 'rusters' have been reported in journals of Industrial Medicine (Collins, 1957; Buckley & Lewis, 1960), but many of the experienced dermatologists and factory doctors we questioned had either never heard of the condition or knew nothing of its cause.

We here describe two 'rusters' we have recently treated, and review previous work regarding the cause and detection of the condition.

CASE REPORTS

Case 1 was a 30-year-old man who had worked for some years as a lorry driver without any symptoms. In 1974, he started a training course in electrical engineering and soon afterwards noticed that any

Reprint requests to Dr J.L.Burton, Bristol.

polished metal he handled became tarnished in a few days. He had no other symptoms and he was normal on examination. He did not appear to sweat excessively, either at rest or on exertion. His father, who worked as an Electronics Engineer, had never noticed any tendency for his own work to become rusty. There was no family history of chest infections or malabsorption to suggest fibrocystic disease.

The patient was asked to bring for inspection a metal sheet he had handled, and the corrosion was noted to start as a series of linear pin-point brown specks where his finger tips had rested. The specks of rust were arranged in a 'finger print' pattern which appeared to correspond to the sites of the pores of the sweat glands, which open onto the crest of the dermatoglyphic ridges. The phenomenon was not confined to the hands, for similar linear rusting could be produced by the soles of the feet (Fig. 1).

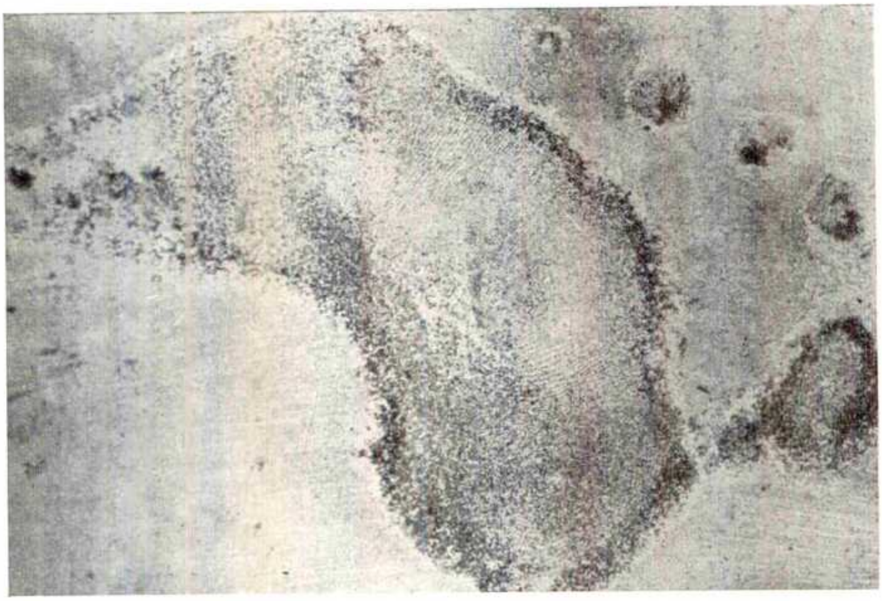


FIGURE 1. A corroded footprint produced by the 'ruster' (Case 1) standing for a few seconds on a metal plate. Note that the corrosion corresponds to the opening of the sweat ducts on crests of the dermatoglyphic ridges.

Sweat samples were collected on filter papers from the axilla and palm for analysis of sodium chloride concentrations. Sweating from the axilla was provoked by vigorous exercise, and palmar sweating was provoked by challenging the patient with mental arithmetic tests. The chloride contration of the axillary sweat was 190 mmol/l and that of the palmar sweat was considerably higher, (around 1000 mmol/l) but the sample was too small for accurate analysis. The normal upper limit for adults in the same laboratory was 80 mmol/l.

Simple therapeutic measures were advised and these included a high fluid intake, the wearing of cool clothing at work, frequent hand washing with the use of a barrier cream, and cleansing of the metal with trichloroethylene immediately after handling. These measures produced a definite improvement and he was able to continue with his training course.

Case 2 was a 21-year-old man who had been an apprentice tool-maker since leaving school. Soon after starting work his employer had complained that any metal work he handled quickly became rusty. His health was excellent, but he had had a transient Bell's palsy some years previously. Since the complaint about his 'rusting', he felt that he sweated excessively compared with his colleagues. On examination he was normal, and did not appear to sweat excessively.

No investigations of his sweat chloride were performed. Treatment with probanthine 15 mg three times daily and a variety of topical antiperspirants produced no improvement in the 'rusting' tendency.

DISCUSSION

Rusting of iron and steel occurs readily in the presence of both atmospheric oxygen and a solution of an electrolyte such as sodium chloride. If a drop of electrolyte solution (e.g. sweat) is placed on iron or steel and there is free exposure to air, rusting starts after a short period in which a concentration gradient for the dissolved oxygen becomes established. The drop is rich in oxygen at the surface and poorer in oxygen at the centre and this concentration gradient enables an electrolytic cell action to start within the drop (Fig. 2). The anode reaction at the centre of the drop is:

$$\text{Fe (solid)} \rightarrow \text{Fe}^{2+} + 2\text{e}^-$$

The cathode reaction at the edge of the drop is:

$$\tfrac{1}{2}\text{O}_2 + \text{H}_2\text{O} + 2\text{e}^- \rightarrow 2\text{OH}^-$$

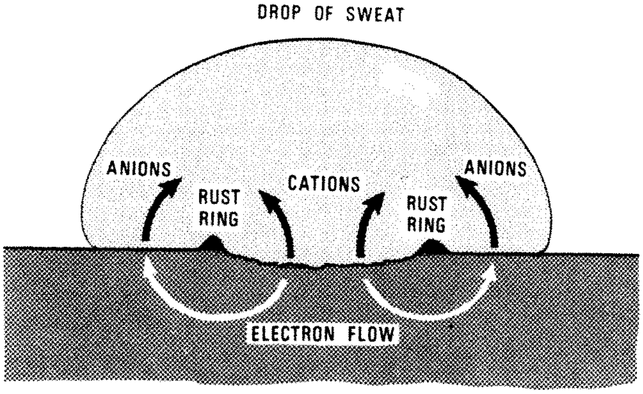


FIGURE 2. Mechanism of corrosion of iron by a drop of sweat. Anodic attack occurs at the centre of the drop but the periphery is spared due to the ready access of oxygen to the metal (diagram modified from Evans, 1963).

In between the anode and cathode Fe^{2+} and OH^- react to give $Fe(OH)_2$ which is rapidly oxidized by oxygen to brown rust, $Fe_2O_3 \cdot n\,H_2O$ (Evans, 1963). Sodium chloride is not involved in the process except to carry the electrical current within the drop of water. Traces of organic acids in sweat could also play a part by helping to break the normal oxide film on the surface of iron or steel allowing the corrosion to begin more quickly.

Collins (1957) has shown that the production of metal corrosion by sweat depends mainly upon the chloride content of the sweat. He measured the degree of corrosion gravimetrically following exposure of the metal to artificial sweat solutions of varying composition and the corrosion was found to be directly proportional to the sodium chloride concentration of the solution used. Most of the other components of sweat (e.g. urea and potassium) had no effect on the amount of corrosion produced, but the addition of lactic acid produced a small increase in corrosion, and normal sweat has a relatively high lactate content (Fishberg & Bierman, 1932). Reduction of the sweat pH was found to produce a small increase in corrosive action, and Eisler & Faigen (1954) have also shown that acidic sweat will corrode more readily than alkaline sweat. From the practical point of view, however, the acidity of sweat is much less important than its chloride concentration.

Collins found that the rate of sweat secretion also influences the amount of corrosion produced. He studied eight normal subjects chosen at random and found that four of these were capable of causing metal to rust within 1½ hours, and these four subjects had the highest rate of sweat production. It is generally believed by workers in the engineering industry that women are more likely to be 'rusters' than men (Buckley & Lewis, 1960), but Collins was unable to obtain any experimental validation of this idea from his small study.

The sweat chloride concentration varies considerably from person to person, and though there is minor fluctuation from day to day, the rate of chloride excretion in a given individual is relatively constant (Darling, 1948). The factors which regulate the rate of chloride excretion by palmar sweat are not fully understood, and the literature is conflicting, partly because few workers have measured both the concentration of chloride and the rate of sweat production. Robinson & Robinson (1954) reported that the chloride concentration varied directly with the rate of sweat excretion, but Lobitz & Osterberg (1947) found that in profuse palmar sweating the chloride concentration was lower than in scanty intermittent sweating. The effect of environmental temperature on the chloride content of sweat is also controversial. It is generally accepted that palmar sweating is mainly increased by emotional rather than thermal stimulation, but the rate of palmar sweat production is not entirely unaffected by environmental temperature, even though the effect is not mediated by direct thermal stimulation of the glands. The rate of sweat production of the palms decreases during heat-induced sweating (Darling, 1948), and basal levels of palmar sweating in females are lowest during the summer months (MacKinnon & MacKinnon, 1956). Kuno (1956) on the other hand reported that at low temperatures, appropriate mental stimuli no longer caused an increase in palmar sweating, and Collins (1957) agreed that palmar sweat production was inhibited at low laboratory temperatures. Johnson, Pitts & Consalazia (1944) reported that the chloride concentration of thermally induced sweating increased with a rise in skin temperature, even on the palms.

A simple screening test to detect 'rusters' has been described by Buckley & Lewis (1960). Glass plates coated with a thin layer of salt-free gelatin impregnated with silver nitrate and potassium chromate are used to allow the chloride concentration of the sweat to be measured chromatographically. The subject washes and dries his hands, and after 20 minutes presses his palm gently on the gelatin plate. The choride ion produces a whitish (silver chloride) to yellowish (sodium chromate) colour against the red background of the plate, and a control plate using known concentrations of chloride ion is used to facilitate interpretation of the test. The subjects can then be placed in one of four groups, according to the colour change noted, as follows:

Group 1. Less than 50 mmol/l (mEq/L) of chloride ion.
Group 2. 50–100 mmol/l (mEq/L) of chloride ion.
Group 3. 100–150 mmol/l (mEq/L) of chloride ion.
Group 4. 150 mmol/l (mEq/L) and above of chloride ion.

Buckley & Lewis (1960) tested the ability of their subjects to corrode polished mild steel plates, and they found that subjects in Group 1 caused no corrosion, Group 2 caused no corrosion or just-detectable corrosion, Group 3 produced corrosion which varied from just-detectable to severe, and Group 4 subjects caused corrosion in every test. All the subjects who were known from previous observations to be 'rusters' were found to fall into Group 4 by this screening test, and Buckley & Lewis (1960) suggested that all staff required to work in critical areas should be screened in this way prior to employment. The test can also be used to identify 'rusters' in workshop areas where corrosion has become a problem.

The management of the individual 'ruster' is not easy. The literature quoted above regarding the control of palmar sweat chloride content is conflicting, but it has been shown that the higher the skin surface temperature, the higher is the chloride content of the surface film (Darling, 1948). The affected worker should avoid sweating as much as possible by wearing cool clothes and keeping the environmental temperature low, if possible. Excessive salt intake should be avoided, since at a constant rate of sweating the chloride concentration parallels the dietary salt intake (Locke *et al.*, 1951). A low-salt diet can reduce the sweat chloride, especially under conditions of heavy thermal sweating (McCance, 1938; Weiner & van Heyningen, 1952) but severe dietary restriction is inadvisable in such circumstances because of the risk of hyponatraemia.

Frequent washing of the hands helps to remove the sodium chloride from the skin surface, and a barrier cream may also help. Collins (1957) found that at high sweat rates corrosion was reduced but not prevented by a silicone emulsion cream or greasy barrier cream with a petroleum jelly base. The worker should understand too that his sweat must be removed from the metal immediately after he has handled it, as corrosion begins very rapidly. Corrosion will still occur if oil is applied after contamination of the metal surface (Huff, 1921) and the parts can be protected by grease only after all traces of sweat have been removed. Several suitable cleaning fluids have been described (Eisler & Faigen, 1954) including 95% methanol in water, and an aqueous alkaline processing fluid (BS1133, 1953).

The effect of the various therapeutic measures aimed at reducing palmar hyperhidrosis does not seem to be recorded in the literature. Neither of our patients responded to oral probanthine, and other measures such as iontophoresis of tap-water and cervical sympathectomy were not attempted.

The range of normal values for sweat sodium levels in the adult are ill-defined (Anderson & Freeman, 1960). Earlier workers suggested that normal values ranged from 52 to 700 mg NaCl per 100 ml (9–120 mmol/l) (Lobitz & Ostenberg, 1947; Darling, 1948), and more recently Peterson (1959) took 70 mmol/l as the normal upper limit. Even this may be too high, as Wood *et al.* (1959) found that none of the fifty adults they studied had a level higher than 50 mmol/l and Smoller & Hsia (1959) found that the level exceeded 50 mmol/l in only three of sixty-four subjects. A level in excess of 70 mmol/l in older children is usually taken to offer strong evidence of fibrocystic disease, but no firm boundary exists and higher levels sometimes occur in apparently normal children (McKendrick, 1962).

The observation that 'rusters' are characterized by an increased excretion of salt in the sweat suggests the possibility that some 'rusters' might have fibrocystic disease of the pancreas, which is reliably detected by the finding of a high sweat sodium (di Sant 'Agnese, 1957). Both of our patients were in excellent health and Buckley & Lewis (1960) found no history of fibrocystic disease of the pancreas in their four patients or in their families. The possibility remains however that some 'rusters' might have a 'forme fruste' of fibrocystic disease, or might share a linked genetic abnormality. A similar suggestion has been made for some other conditions in which surveys have shown an increased incidence of individuals with high sweat chloride excretion rates. These include chronic bronchitis (Karlisch & Tarnovsky, 1960), pulmonary emphysema (Wood *et al.*, 1959) and some allergic states (Hsia *et al.*, 1958).

It is unlikely that 'rusters' represent a heterozygote state of fibrocystic disease, since although siblings and parents of children with this disease show a slightly higher sweat sodium level than normal, the average increase is only about 10 mmol/l (McKendrick, 1962), and this small increase in palmar sweat chloride would not be sufficient to produce a 'ruster'.

ACKNOWLEDGMENT

Thanks are due to Dr P.L.Timms, Reader in Inorganic Chemistry, Bristol University, for advice on the chemistry of rusting.

REFERENCES

ANDERSON, C.M. & FREEMAN, M. (1960) Sweat test results in normal persons of different ages compared with families with fibrocystic disease of the pancreas. *Archives of Diseases of Childhood*, **35**, 381.

BS1133 PACKAGING CODE, SECTION 6 (1953) *Temporary prevention of corrosion of metal surfaces*. British Standards Institution, London.

BUCKLEY, W.R. & LEWIS, C.E. (1960) The 'ruster' in industry. *Journal of Occupational Medicine (Baltimore)*, **2**, 23.

COLLINS, K.J. (1957) The corrosion of metal by palmar sweat. *British Journal of Industrial Medicine*, **14**, 191.

DARLING, R.C. (1948) Some factors regulating the composition and formation of human sweat. *Archives of Physical Medicine*, **29**, 150.

DI SANT 'AGNESE, P.A. (1957) Exocrine gland dysfunction in cystic fibrosis of the pancreas. *Acta Paediatrica (Stockholm)*, **46**, 51.

EISLER, S.J. & FAIGEN, H.L. (1954) Investigation of synthetic fingerprint solutions. *Corrosion*, **10**, 237.

EVANS, U.R. (1963) *An Introduction to Metal Corrosion*, p. 31. E.Arnold, London.

FISHBERG, E.H. & BIERMAN, W. (1932) Excretion of lactic acid in sweat. *Proceedings of the Society of Experimental Biology and Medicine*, **29**, 102.

HUFF, W.J. (1921) Corrosion protective action of certain colloidal solutions. *Chemical and Metallurgical Engineering*, **25**, 865.

HSIA, D.Y.Y., DRISCOLL, S.G., GREENBERG, D., LEE, T.C. & LANOFF, G. (1958) Abnormal sweat electrolytes in patients with allergies. *Diseases of Childhood*, **96**, 685.

JOHNSON, R.E., PITTS, G.C. & CONSALAZIA, F.C. (1944) Factors influencing chloride concentration in human sweat. *American Journal of Physiology*, **141**, 575.

KARLISCH, A.J. & TARNOVSKY, A.L. (1960) Mucoviscidosis as a factor in chronic lung disease in adults. *Lancet*, **ii**, 514.

LOBITZ, W.C. & OSTERBERG, A.E. (1947) Chemistry of palmar sweat: II Chloride. *Archives of Dermatology and Syphilology*, **56**, 462.

LOCKE, W., TALBOT, N.B., JONES, H.S. & WORCESTER, H. (1951) Studies on the combined use of measurements of sweat electrolyte composition and rate of sweating as an index of adrenal cortical activity. *Journal of Clinical Investigation*, **30**, 325.

MCCANCE, R.A. (1938) The effect of salt deficiency in man on the volume of the extracellular fluids, and on the composition of sweat, saliva, gastric juices and cerebrospinal fluid. *Journal of Physiology (London)*, **92**, 208.

MCKENDRICK, T. (1962) Sweat sodium levels in normal subjects in fibrocystic patients and their relatives, and in chronic bronchitic patients. *Lancet*, **i**, 183.

MACKINNON, P.C.B. & MACKINNON, I.L. (1956) Seasonal sweating. *Nature (London)*, **177**, 799.

PETERSON, E.M. (1959) Consideration of cystic fibrosis in adults with a study of sweat electrolyte values. *Journal of the American Medical Association*, **171**, 1.

ROBINSON, S. & ROBINSON, A.H. (1954) Chemical composition of sweat. *Physiology Review*, **34**, 202.

SMOLLER, M. & HSIA, D.Y.Y. (1959) Studies on the genetic mechanism of cystic fibrosis of the pancreas. *Diseases of Childhood*, **98**, 277.

VERNON, W.H.J. (1935) A laboratory study of the atmospheric corrosion of metals. *Transactions of the Faraday Society*, **31**, 1668.

WEINER, J.S. & VAN HEYNINGEN, R. (1952) Salt losses of men working in hot environments. *British Journal of Industrial Medicine*, **9**, 56.

WOOD, J.A., FISHMAN, A.P., REEMTSMA, K., BARKER, H.G. & DI SANT 'AGNESE, P.A. (1959) A comparison of sweat chlorides and intestinal fat absorption in chronic obstructive pulmonary emphysema and fibrocystic disease of the pancreas. *New England Journal of Medicine*, **260**, 951.

British Journal of Dermatology (1976) 95, 423.

Pharmacology and Treatment

Proprietary hydrocortisone creams

VASOCONSTRICTOR ACTIVITIES AND BIO-AVAILABILITIES OF SIX PREPARATIONS

B.W.BARRY AND R.WOODFORD

School of Pharmacy, Portsmouth Polytechnic, King Henry I Street, Portsmouth PO1 2DZ

Accepted for publication 6 February 1976

SUMMARY

Six proprietary hydrocortisone creams were evaluated for vasoconstrictor activities and bio-availabilities using an occluded blanching test. Statistical analysis showed a significant difference between the formulations. Dioderm, containing 0·1% hydrocortisone, was significantly more active than the 1% hydrocortisone creams—Alphaderm, Calmurid HC, Efcortelan and Vioform-Hydrocortisone. There was no significant difference between Dioderm and Dioderm C. Unlike creams containing more potent corticosteroids the hydrocortisone formulations apparently failed to produce steroid reservoirs in the stratum corneum as assessed by the blanching response.

Because of the possible adverse side effects of highly potent fluorinated topical corticosteroids there is renewed interest in hydrocortisone preparations. Almeyda & Burt (1974) reported a study of atopic eczema treatment with 1% hydrocortisone in a stabilized urea-containing base. Whitefield & McKenzie (1975) showed that a cream containing 0·1% hydrocortisone dissolved in propylene glycol (Dioderm) produced vasoconstriction and was clinically superior in eczema treatment to Hydrocortisone Cream BPC. The aim of the present study was to compare the vasoconstrictor activities and, by inference, the clinical anti-inflammatory efficacies and bio-availabilities of selected modern hydrocortisone-containing creams. The preparations were Dioderm, Dioderm C (both containing 0·1% hydrocortisone), Alphaderm, Calmurid HC, Efcortelan 1% and Vioform-Hydrocortisone (all containing 1% hydrocortisone). Efcortelan 1% complied with the specification for Hydrocortisone Cream BPC.

MATERIALS AND METHODS

The 6 h occluded blanching test with subsequent re-occlusion was employed and results were statistically analysed as previously described (Barry & Woodford, 1974, 1975).

RESULTS AND DISCUSSION

Fig. 1 shows vasoconstrictor profiles for the hydrocortisone preparations while Table 1 lists the blanching responses for each formulation.

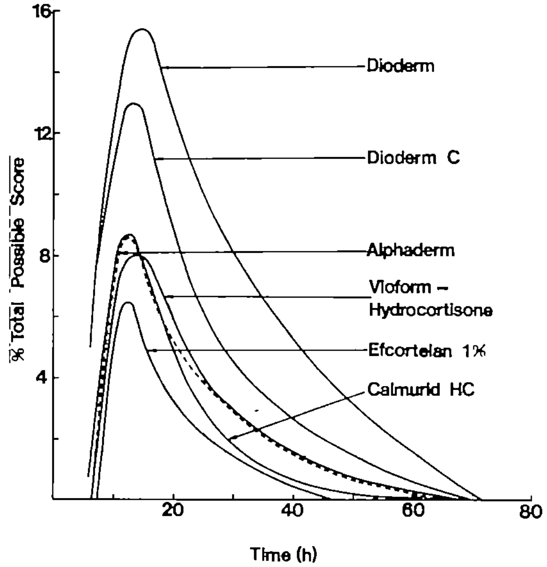


FIGURE 1. Percentage of the total possible score as a function of time for formulations indicated.

TABLE 1. Blanching response to proprietary hydrocortisone preparations

Cream	Steroid and % w/w	Area under the curve %×h*	Summed % total possible score†	Tm/10 mean value, square root transformation‡
Dioderm	Hydrocortisone, 0·1	434	66	2·21
Dioderm C	Hydrocortisone, 0·1	289	57	1·95
Vioform-Hydrocort.	Hydrocortisone, 1·0	174	23	1·20
Alphaderm	Hydrocortisone, 1·0	173	26	1·13
Calmurid HC	Hydrocortisone, 1·0	156	24	1·09
Efcortelan, 1%	Hydrocortisone, 1·0	93	15	0·76

* Obtained by planimetry of the blanching profile. † The percentage of the total possible scores summed for all volunteers over all reading times. ‡ The Tm/10 value is the square root transformation of sum of scores (Tm) divided by the number of volunteers (10). The minimum significant range value k = 0·99 ($P = 0·05$), i.e. if the Tm/10 values of two preparations differ by more than 0·99 there is a significant difference between those preparations.

Dioderm and Dioderm C produced greater percentage total possible scores and area under the curve values than the other hydrocortisone preparations, even though Dioderm and Dioderm C contained hydrocortisone at one-tenth of the concentration present in the other products. A ranking of the formulations using the area under the curve bio-availability parameter showed Dioderm and

Dioderm C to be better than the other preparations. The rank order produced by this approach was the same as that given by the 'square root transformation' sum of responses employed in the statistical analysis. Statistical analysis indicated that there was no significant difference ($P<0{\cdot}05$) between Dioderm and Dioderm C. Furthermore, Dioderm was significantly more active ($P<0{\cdot}05$) in the blanching test than Alphaderm, Calmurid HC, Efcortelan 1% and Vioform-Hydrocortisone, all of which were statistically equivalent.

Re-occlusion of the application sites for 12 h, 8 days after commencement of the experiment, failed to elicit blanching by any preparation indicating that no demonstrable hydrocortisone reservoir formed in the stratum corneum.

REFERENCES

ALMEYDA, J. & BURT, B.W. (1974) Double-blind controlled study of treatment of atopic eczema with a preparation of hydrocortisone in a new drug delivery system versus betamethasone 17-valerate. *British Journal of Dermatology*, **91**, 579.

BARRY, B.W. & WOODFORD, R. (1974) Comparative bio-availability of proprietary topical corticosteroid preparations; vasoconstrictor assays on thirty creams and gels. *British Journal of Dermatology*, **91**, 323.

BARRY, B.W. & WOODFORD, R. (1975) Comparative bio-availability and activity of proprietary topical corticosteroid preparations; vasoconstrictor assays on thirty-one ointments. *British Journal of Dermatology*, **93**, 563.

WHITEFIELD, M. & MCKENZIE, A.W. (1975) A new formulation of 0·1% hydrocortisone cream with vasoconstrictor activity and clinical effectiveness. *British Journal of Dermatology*, **92**, 585.

British Journal of Dermatology (1976) 95, 427.

Effect of 1% cyproterone acetate in Cetomacrogol Cream BPC (Formula A) on sebum excretion rate in patients with acne

R.J.PYE, J.L.BURTON AND J. I. HARRIS*

Department of Dermatology, Bristol Royal Infirmary, Bristol, and
* School of Pharmacy and Pharmacology, University of Bath, Bath

Accepted for publication 26 March 1976

Acne vulgaris has been shown to be associated with seborrhoea, and seborrhoea is androgen dependent. Cyproterone acetate is an anti-androgen which, when given orally, reduces sebum excretion (Burton, Laschet & Shuster, 1973) and improves acne (Winkler, 1968; Leo-Rossberg *et al.*, 1971; Hammerstein *et al.*, 1975). It has not yet been widely used in acne, as it has unacceptable side-effects such as loss of secondary sexual characteristics and libido in men and animal experiments suggest it may produce feminization of the male fetus in pregnant women. Topical application has been investigated in patients with acne by Cunliffe, Shuster & Cassels-Smith (1969), but there was no significant decrease in sebum excretion or improvement in the patients' acne. Dimethylsulphoxide (DMSO) 50% was used to improve penetration of the anti-androgen, however, and doubt has since been raised about the suitability of DMSO as the vehicle (Cunliffe & Cotterill, 1975). We here report the effect of topical 1% cyproterone acetate in suspension in Cetomacrogol cream BPC (Formula A).

Fourteen patients, nine female aged 15–27 years and five male aged 18–24 years, with moderate to severe acne were studied. The sebum excretion rate (SER) was measured by the gravimetric method of Strauss & Pochi (1961) as modified by Cunliffe & Shuster (1969). SER was measured in all patients before treatment. The cream was applied twice a day to both sides of the forehead. Measurements were repeated in all patients after 4 weeks, and in eight patients after 8–12 weeks' treatment.

As can be seen from Table 1, there was no significant difference in the SER before and after treatment. None of the patients showed any marked improvement in their acne, and no side-effects were reported.

TABLE 1. SER before and after topical cyproterone acetate

	Sebum excretion rate (μg/cm^2/min) mean ± s.e.			
	Male	No.	Female	No.
Before	1·42 ± 0·23	5	1·05 ± 0·17	9
After 4 weeks	1·32 ± 0·28	5	1·14 ± 0·15	9
After 8–12 weeks	1·40 ± 0·16	4	1·10 ± 0·15	4

Topical anti-androgens offer a logical approach to the treatment of acne and hirsutism, but the main problem is in finding a suitable vehicle to allow adequate percutaneous penetration. We have found no effect with 1% cyproterone acetate in Cetomacrogol cream BPC used for up to 3 months, whereas systemic cyproterone acetate reduces seborrhoea in acne patients within 2 months (Leo-Rossberg *et al.*, 1971). A previous trial using 1% cyproterone acetate in solution within a polyoxyethylene base (Macrogol Ointment BPC) was abandoned because the preparation caused severe pruritus at the site of application after a few days' use.

ACKNOWLEDGMENT

Thanks are due to Dr Harcus of Schering AG for a gift of cyproterone acetate.

REFERENCES

Burton, J.L., Laschet, U. & Shuster, S. (1973) Reduction of sebum excretion in man by the anti-androgen cyproterone acetate. *British Journal of Dermatology*, **89**, 487.

Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 607.

Cunliffe, W.J., Shuster, S. & Cassels-Smith, A.J. (1969) The effect of topical cyproterone acetate on sebum secretion in patients with acne. *British Journal of Dermatology*, **81**, 200.

Cunliffe, W.J. & Cotterill, J.A. (1975) *The Acnes*, p. 120. W.B.Saunders, Philadelphia.

Hammerstein, J., Meckies, J., Leo-Rossberg, L., Moltz, L. & Zielske, F. (1975) Use of cyproterone acetate (C.P.A.) in the treatment of acne, hirsutism and virilism. *Journal of Steroid Biochemistry*, **6**, 827.

Leo-Rossberg, I., Laur, S., Zielske, F. & Hammerstein, J. (1971) Reversed sequential therapy of hirsutism using cyproterone acetate. *Acta Endocrinologica (Kbh)* Suppl. **152**. Abstract 14.

Strauss, J.S. & Pochi, P.E. (1961) The quantitative gravimetric determination of sebum production. *Journal of Investigative Dermatology*, **36**, 293.

Winkler, K. (1968) La valeur des anti-androgènes en dermatologie. *Annales de Dermatologie et de Syphiligraphie*, **95**, 147.

British Journal of Dermatology (1976) 95, 429.

Case Reports

Disseminated superficial actinic porokeratosis*

ANALYSIS OF AN AFFECTED FAMILY

DONALD J.PIROZZI† AND ALBERT ROSENTHAL

Division of Dermatology, Department of Medicine, Hahnemann Medical College, Philadelphia, PA. 19102, U.S.A.

Accepted for publication 23 January 1976

SUMMARY

A family is analysed in which disseminated superficial actinic porokeratosis (DSAP) occurred in five members. All three children of one generation are affected. Clinical, histopathological and genetic aspects of DSAP are discussed. Disseminated superficial actinic porokeratosis appears to be a clinical variant of porokeratosis of Mibelli. Since DSAP is a genodermatosis inherited as an autosomal dominant it can occur in any geographical location; however excess sunlight can definitely exacerbate the condition.

Disseminated superficial actinic porokeratosis (DSAP) is a genodermatosis with a typical histopathological picture and is not rare, at least in locations where patients are exposed to a large amount of sunlight. It has been stated that the disease is inherited as an autosomal dominant with reduced penetrance at young ages (Anderson & Chernosky, 1969). Recently an investigation of a family was made in which five members were affected. The disease began to manifest itself in three of these patients during adolescence.

METHOD

Three of the patients were interviewed for familial information concerning who was affected. Fig. 1 shows the results of that inquiry.

CASE REPORTS

Case 1. This patient is a 70-year-old white male and the father of the following three cases. He has always lived in the North-eastern part of the United States. Early in his life he noted small, hypopigmented lesions on the sun exposed areas of his body. He was unavailable for examination.

Case 2. The eldest daughter is a 43-year-old white female who has lived in the North-eastern section of the United States all of her life. Since the age of 10 she has had hypopigmented, scaling lesions on her arms and legs. At present hundreds of 1–7 mm scaly, annular, erythematous lesions are noted on the dorsa of both hands and on both arms up to the elbow (Fig. 2). The patient claims that all lesions worsen when exposed to sunlight and then become somewhat pruritic. A biopsy specimen taken from a typical lesion was consistent with DSAP.

---

* Presented in part before the 124th Annual Convention of the American Medical Association, Section on Dermatology, Atlantic City, N.J., 16 June, 1975.

† Dr Pirozzi is currently Assistant Professor of Medicine (Dermatology) at Emory University School of Medicine, Atlanta, Georgia, U.S.A.

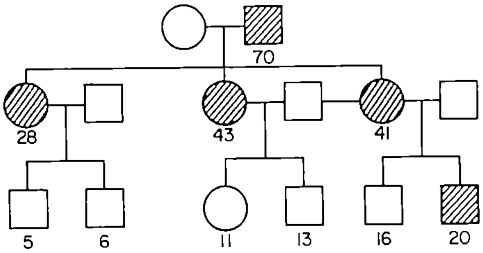


FIGURE 1. Pedigree of affected family. Hatched areas, affected; circles, female; squares, male.

Case 3. The patient is a 41-year-old white female who has always lived in the North-east United States. Since the age of 15 she has noted 'white marks' on the sun exposed area of her body. When exposed to sunlight the lesions become markedly erythematous and pruritic. In recent years the patient has noted progressively increasing numbers of lesions. Physical examination reveals several hundred erythematous, annular, scaly lesions of the arms, legs and 'V' of the neck. This patient was the most affected member of the family and was noted to have lesions as high as the upper parts of both legs. Histopathological examination of the lesions revealed DSAP.

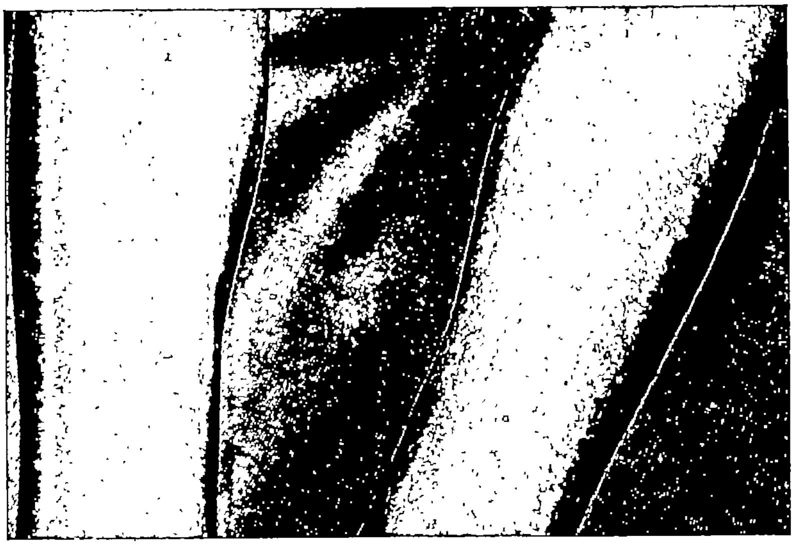


FIGURE 2. Lesions of disseminated superficial actinic porokeratosis on flexor aspects of arms (Case 2).

Case 4. The patient is a 28-year-old white female who has always lived in the North-eastern section of the United States. Since the age of 15 she has noted 'white marks' on her arms and legs which seem to worsen in sunlight. These marks are minimally pruritic. Physical examination reveals hundreds of 1–5 mm annular, scaly, hypopigmented lesions on the dorsa of both hands and arms, and on the anterior tibial areas of both legs. This patient tries to avoid sunbathing since it has a deleterious effect on her skin and has thus not noted an increase of lesions in recent years. Histopathological examination revealed changes typical of DSAP.

Case 5. The patient is a 21-year-old white male (the son of Case 2) and has recently noted the appearance of multiple, scaling, hypopigmented lesions on both lower legs which are asymptomatic. He has no lesions elsewhere on the body and has always lived in the North-eastern section of the United States. The patient was unavailable for examination.

Histopathology

Histopathological diagnosis of this entity depends upon finding the typical cornoid lamella. This finding is similar to that found in porokeratosis of Mibelli. The cornoid lamella is a parakeratotic column that stains lighter than the adjacent stratum corneum with haematoxylin and eosin. Beneath it the granular layer is absent and the malphigian layer usually atrophic, although it can be slightly acanthotic (Fig. 3). Vacuolated cells can be seen beneath the cornoid lamella and patches of lymphocytes and histiocytes may be present in the adjacent dermis. Other lesions can show hyperkeratosis, acanthosis, vascular proliferation and dilatation, abundant cellular infiltrate and an increase of dermal connective tissue (Chernosky & Freeman, 1967).

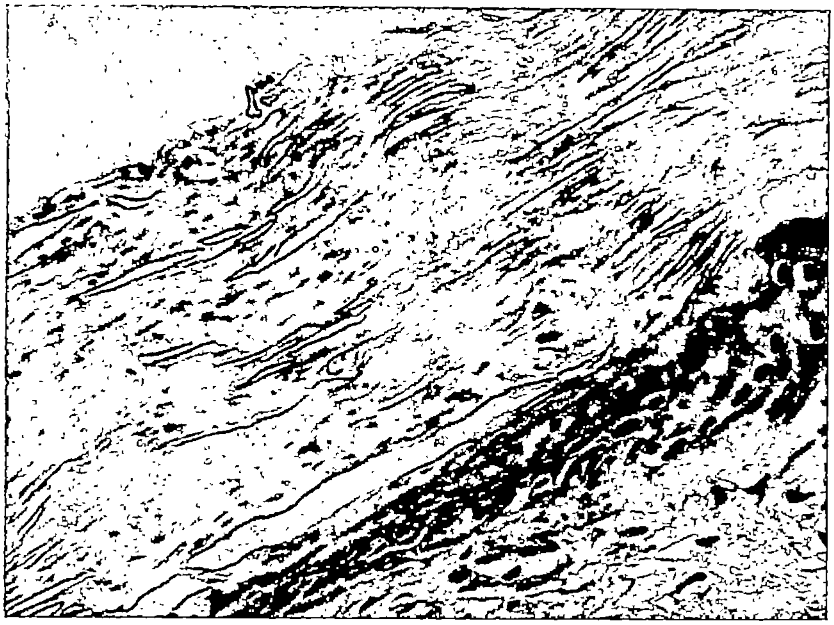


FIGURE 3. Close-up of cornoid lamella with parakeratotic column of stratum corneum, absence of granular layer beneath the cornoid lamella and atrophic malpighian layer (H & E, × 45).

COMMENT

Mibelli (1889) first described porokeratosis thinking it represented a disturbance in the keratinization of the acrosyringial portion of the eccrine sweat ducts. The patient developed a lesion on the back of the right hand at the age of 2, which progressively enlarged and multiplied. Later on in the course of the disease, similar lesions appeared on the back of the left hand. The lesions were asymptomatic. In 1928 Lain reported three cases of porokeratosis having multiple lesions. In 1934 Freund described a variety of porokeratosis which was multiple, superficial, and smaller than that originally described by Mibelli. In 1937 Andrews described a case similar to Freund's and designated the type of porokeratosis as 'disseminated and superficial type'. In 1966 Chernosky drew attention to the fact that the multiple, symmetrical superficial type of porokeratosis was more common than previously suspected. He also noted that in his original twelve cases, the lesions were bilateral and scattered over exposed areas of the skin, and especially in those prone to sun exposure. In 1967 Chernosky & Freeman reported thirty-one patients with what they called disseminated superficial actinic porokeratosis. In this study they determined that although histopathologically the disease resembles porokeratosis of Mibelli, clinically it is different. It was also noted (Chernosky & Freeman, 1967; Chernosky & Anderson, 1969) that natural and experimental UV light was significant in the genesis of this disease, and that the lesions often clinically and histopathologically resemble actinic keratoses. The lesions of porokeratosis of Mibelli are usually larger and have a predilection for the extremities, face and neck, although many patients have lesions of the mucous membranes. Genetic factors were assumed to be different, since the original case of porokeratosis of Mibelli (and subsequent cases) arose early in life, while most of Chernosky's cases arose in males or females after the third decade and no earlier than the age of 16. Thus, it was determined that DSAP has an autosomal dominant inheritance with reduced penetrance at younger ages (Anderson & Chernosky, 1969) while porokeratosis of Mibelli has an autosomal dominant inheritance.

Reed & Leone (1970) and Shelley (1974) have suggested that porokeratosis of Mibelli may represent a clinical expression of a UV light-triggered mutant clone of cells in the epidermis. Since both porokeratosis of Mibelli and DSAP are related histopathologically to actinic keratosis, a hypothesis can be made which states that the so-called abnormal clones in porokeratosis are more sensitive to sunlight (UV light) than is normal tissue and may develop into clinical lesions after light stimulation. If abnormal clones are a feature of either type of porokeratosis, an increased susceptibility to the development of neoplasia would not be surprising. As expected, several cases of squamous cell carcinoma developing in lesions of biopsy-proven porokeratosis of Mibelli have been noted (Johnston, 1958; Savage, 1964). However no reported cases of DSAP have eventuated into squamous cell carcinoma.

The cases reported in this paper represent an unusual circumstance in that all members of one generation were affected with DSAP and give histories of purposeful avoidance of sunlight. The initial lesions also developed relatively early in the life of these patients (at the age of 10 in Case 2). One can assume that if a mutant clone did exist, then if these patients lived in an area of excessive sunlight the clinical lesions would have developed even earlier (as they do in porokeratosis of Mibelli). Thus, it would seem that the only difference between disseminated superficial actinic keratosis and porokeratosis of Mibelli is clinical with both inheritance and histopathology being similar.

REFERENCES

ANDERSON, D.E. & CHERNOSKY, M.D. (1969) Disseminated superficial actinic porokeratosis, genetic aspects. *Archives of Dermatology*, **99**, 408.

ANDREWS, G.C. (1937) Porokeratosis (Mibelli) disseminated and superficial type. *Archives of Dermatology and Syphilology*, **36**, 111.

CHERNOSKY, M.E. (1966) Porokeratosis: report of twelve patients with multiple superficial lesions. *Southern Medical Journal*, **59**, 289.

CHERNOSKY, M.E. & FREEMAN, R.C. (1967) Disseminated superficial actinic porokeratosis (DSAP). *Archives of Dermatology*, **96**, 611.

CHERNOSKY, M.E. & ANDERSON, D.E. (1969) Disseminated superficial actinic porokeratosis, clinical studies and experimental production of lesions. *Archives of Dermatology*, **99**, 401.

FREUND, E. (1934) Unusual case of systematized porokeratosis of Mibelli in a child (minimal form). *Archives of Dermatology and Syphilology*, p. 253. Yearbook Publishers, Chicago.

JOHNSTON, E.N.W. (1958) Porokeratosis of Mibelli with squamous cell carcinoma. *British Journal of Dermatology*, **70**, 381.

LAIN, E.S. (1928) Porokeratosis: Report of three cases. *Urologic Cutaneous Review*, **32**, 383.

MIBELLI, V. (1889) Porokeratosis. In: *International atlas of rare skin diseases* (Ed. by M.A.Morris *et al.*), edn. 27, p. 8. Leopold Voss, Hamburg.

REED, R.J. & LEONE, P. (1970) Porokeratosis—a mutant clonal keratosis of the epidermis. I. Histogenesis. *Archives of Dermatology*, **101**, 340.

SAVAGE, J. (1964) Porokeratosis (Mibelli) and carcinoma. *British Journal of Dermatology*, **76**, 489.

SHELLEY, W.B. (1974) Porokeratosis of Mibelli. In: *Consultations in Dermatology*, II (Ed. by W.B.Shelley), p. 202. W.B.Saunders, Philadelphia.

British Journal of Dermatology (1976) 95, 433.

Unilateral epidermal naevus resembling acanthosis nigricans*

HELEN OLLENDORFF CURTH

Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York, U.S.A.

Accepted for publication 6 February 1976

SUMMARY

A man born with a unilateral epidermal naevus on the right side of the abdomen developed at puberty symmetrical benign acanthosis nigricans in the body folds. The symmetrical eruption later disappeared completely. The unilateral epidermal naevus and the symmetrical eruption showed identical histological features, i.e. those of acanthosis nigricans. At the age of 32 he developed a muco-epidermoid cancer of the left parotid gland. A decision whether the association of unilateral epidermal naevi with malignant tumours occurs with unusual frequency requires (a) a long follow-up of the patient with the unilateral naevus, (b) a differentiation of the unilateral eruption from unilateral malignant acanthosis nigricans and (c) statistical evidence.

History

No instance of similar cutaneous changes as in the patient occurred in his forebears, his 2 sons and 3 grandchildren. His paternal grandmother died of cancer of the breast and his mother at the age of 32 of colloid carcinoma of the rectum with metastases. His father had a pigmented naevus on the right side of the abdomen. A brother, who is 5 years younger than the patient, has a considerable number of naevi.

The patient, who was described at the age of 13 for the first time (Lowenfish, 1934) and in 1936 for the second time (Curth, 1936), was born with a unilateral naevus on the right side of his abdomen. The unilateral eruption extended from the midline of the abdomen over the right side to the spine. It showed hyperpigmented, elevated and verrucous changes. It never changed and increased only proportionately with the body growth.

Histology

The sections (Fig. 1) showed very sharp papillary projections. The depressions between the projections were filled with keratin, which was lamellated. This often formed intraepithelial cysts. Acanthosis was marked. In some places fairly long rete cones were seen projecting into the corium. The epidermis was oedematous. The basal cell layer contained variable amounts of melanin. The corium was the site of a chronic inflammatory reaction. With the Fontana stain large amounts of melanin were seen in the basal layer and appreciable amounts also in the more superficial epidermal layers. Chromatophores were fairly numerous in the corium. *Diagnosis: Epidermal naevus*

At puberty a symmetrical eruption appeared in the axillae (Fig. 2), groins and over large parts of the

---

* Presented at the Dermatological Society of Greater New York, at the April meeting, 1975.

Author's address: 650 West 261st Street, New York, New York 10471, U.S.A.

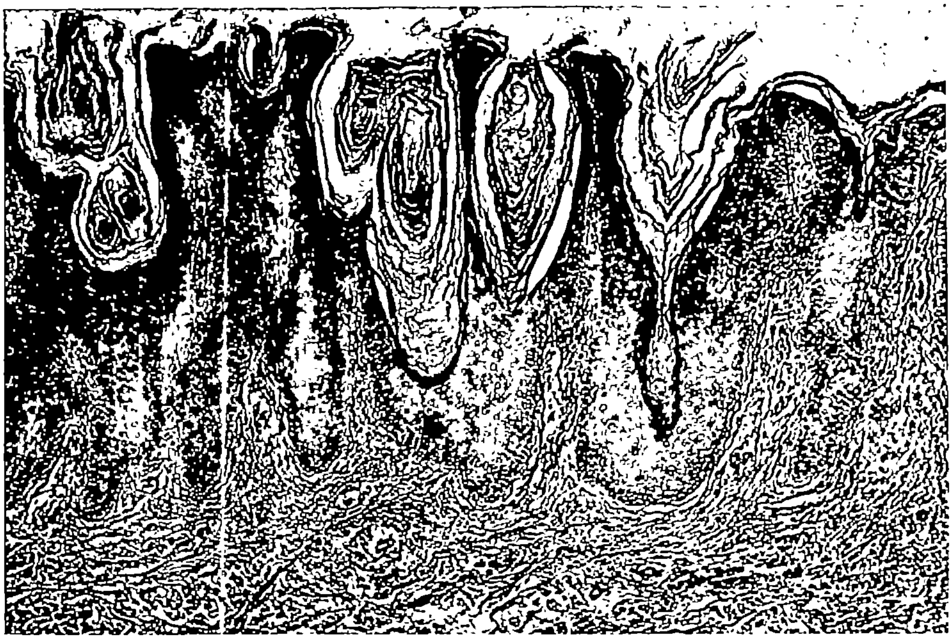


FIGURE 1. Histology of the unilateral naevus. With permission of *Hautarzt*, 15, 435 (1964). Die Probleme der Acanthosis nigricans. Copyright 1964, Springer-Verlag.

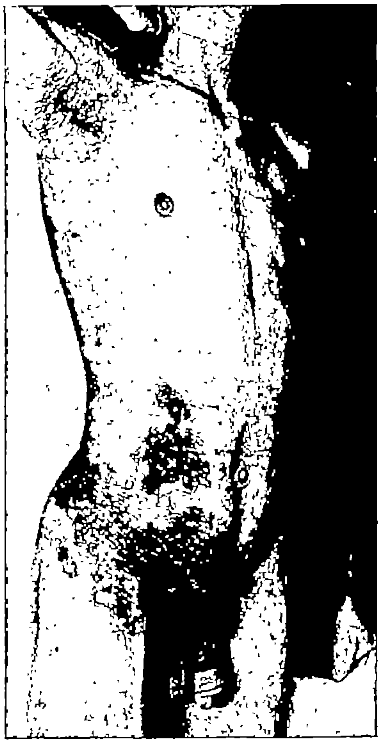


FIGURE 2. Symmetrical eruption in the axillae and on the neck and unilateral eruption on the right side of the abdomen at the age of 14. With permission of the *Archives of Dermatology*, 79, 59 (1959). Genetic studies on acanthosis nigricans. Copyright 1959, American Medical Association.

body. The nipples and the umbilicus showed darkly pigmented and hypertrophic skin. The palms and soles presented irregular areas of dirty-looking hyperkeratoses. The backs of his heels were thickened and scaly. His nails broke easily. The patient also displayed many discrete papillomatous lesions, warty excrescences on the scalp and many pigmented naevi on his trunk.

Histology (Fig. 3)

The epidermis showed numerous sharp projections, consisting of large papillae covered with fairly uniform epithelium. There was marked hyperkeratosis, and the depressions between the projections were filled with lamellated keratin-filled cysts. In some places the papillary outgrowths were replaced by diffuse thickening of the epidermis. The finely fibrillar papillary layer was wide and somewhat conspicuous. *Diagnosis: Acanthosis nigricans*

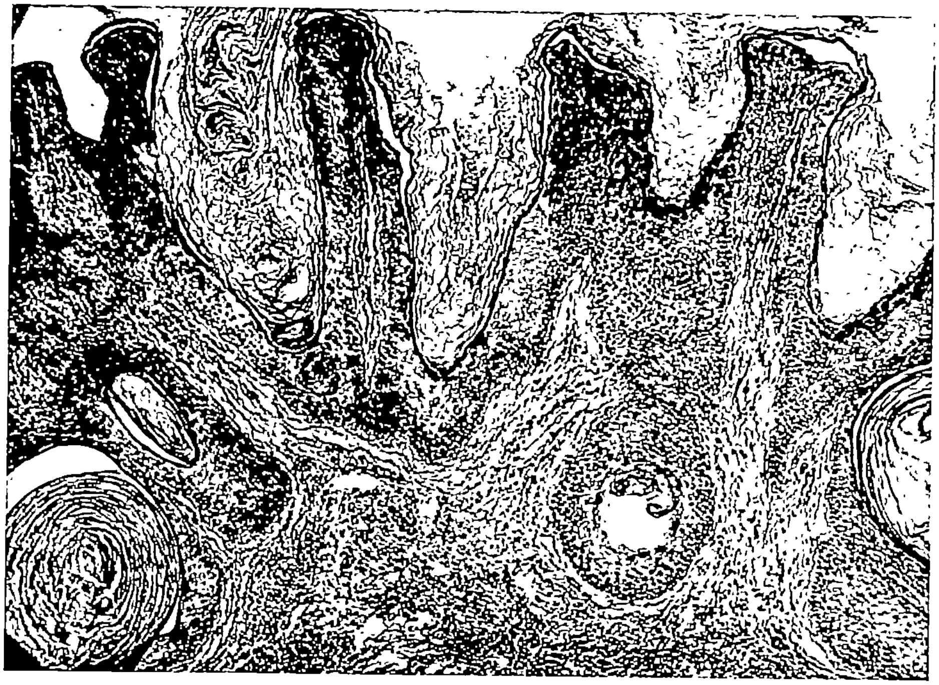


FIGURE 3. Histology of the axillary lesion. With permission of the *Archives of Dermatology and Syphilology*. Benign type of acanthosis nigricans. Copyright 1936, American Medical Association.

The symmetrical eruption disappeared completely, when the patient was between 16- and 17-years-old. At the age of 32 he developed a muco-epidermoid cancer of the left parotid gland. The tumour was removed at Memorial Hospital, New York. Lately, the patient has noticed many new pigmented moles on his body.

Comment

The patient suffered from a unilateral epidermal naevus resembling acanthosis nigricans, benign symmetrical acanthosis nigricans and a muco-epidermoid cancer of the left parotid gland.

This case seems important for two reasons: (1) It showed histological identity of a unilateral epidermal naevus with symmetrical benign acanthosis nigricans although, clinically, the two eruptions showed differences.

It was in this case obvious to consider and easy to prove the histological identity of the unilateral epidermal naevus with benign acanthosis nigricans since the two eruptions occurred in the same patient. This association has to my knowledge not been reported before.

This interesting association invites some speculation as to the relationship of the two conditions to each other. The possibility of the association being fortuitous is remote since it seems improbable that the unilateral naevus, histologically identical with a subsequent symmetrical eruption of a rare dermatosis, occurred by chance.

(2) Another significant feature of this unique case may be that it shows, as do a number of other instances of unilateral naevi, the association with a malignant tumour. Whether malignant tumours occur in patients with unilateral naevi with greater frequency than expected, is difficult to decide since (a) the two conditions develop at different times, (b) the two conditions co-exist without the cancer having a visible effect on the unilateral naevus, and (c) the malignant tumours comprise a multitude of malignant conditions.

It is difficult to decide whether the unilateral epidermal naevus of Case 1 of Pack & Sunderland (1941) represented unilateral malignant acanthosis nigricans. Photographs taken at various intervals suggest that the unilateral condition had spread somewhat and had also involved the left axilla. Subsequently, a left mammary carcinoma developed. Histologically, the unilateral naevus presented according to the photographs of the paper and slides, which I was permitted to study, changes compatible with acanthosis nigricans. A new cutaneous lesion between the two breasts at the age of 13 had a marked inflammatory component. The young woman died at the age of 23 from an adenocarcinoma of the left breast, which had always been smaller than the right one, and with metastases to the left axilla and lungs.

In the case, reported by Halty, Delgado & Volpé (1933) malignant acanthosis nigricans may have begun as a unilateral lesion. The child was born with a unilateral cutaneous condition and showed at the age of 9 months the first signs of an abdominal tumour. It died at the age of 2½ years shortly after laparotomy had revealed a large and inoperable growth. The cutaneous eruption, except for insignificant spreading, remained confined to one side.

In Pack & Sunderland's Case 2 the papillary pigmented naevus, present since birth on the right side of the body, never changed. At the age of 43 the male patient developed a fatal epidermoid cancer of the oesophagus. The naevus remained the same while the tumour, which was not an adenocarcinoma, developed. The unilateral naevus in this instance did not represent malignant acanthosis nigricans.

Holden and Dekaban (1972) found among forty patients with unilateral naevi eight (20·5%) with various cancers (of skin, brain, breast, oesophagus and salivary glands).

Instances of unilateral naevi must be followed up for many years to ascertain if they show an increased potentiality for the association with malignant tumours. Only statistical evidence can decide if the association between unilateral naevi and malignant tumours occurs in these patients unusually often.

REFERENCES

Curth, H.O. (1936) Benign type of acanthosis nigricans. Etiology. *Archives of Dermatology and Syphilis*, **34**, 353.

Halty, A., Delgado, B.C. & Volpé, A. (1933) Acanthosis nigricans. *Revista sud-américana de médicine*, **4**, 189.

Holden, K.R. & Dekaban, A.S. (1972) Neurological involvement in naevus unius lateris and naevus linearis sebaceus. *Neurology*, **22**, 879.

Lowenfish, P. (1934) Acanthosis nigricans (juvenile benign form). *Archives of Dermatology and Syphilis*, **29**, 763.

Pack, G.T. & Sunderland, D.A. (1941) Naevus unius lateris. *Archives of Surgery*, **43**, 341.

British Journal of Dermatology (1976) 95, 437.

Reticulate acropigmentation of Kitamura

W.A.D.GRIFFITHS

St John's Hospital for Diseases of the Skin, Lisle Street, London WC2H 7BJ

Accepted for publication 20 February 1976

SUMMARY

Seven cases of reticulate acropigmentation of Kitamura (RAPK) are described. All the patients were female and the pattern of inheritance suggested an autosomal dominant mode. Histologically, RAPK is characterized by epidermal atrophy, an increased number of basal melanocytes and the absence of pigmentary incontinence in the upper dermis. The present cases, from Asia, Africa and Europe, are the first to be described outside Japan.

A number of pigmentary disorders have until now been assumed to be confined largely to the Japanese. This paper describes seven patients of widely differing ethnic background and skin colour who suffered from an unusual reticulate pigmentary disorder of the extremities. A male patient with reticulate acropigmentation was described in Japan (Kitamura & Akamatsu, 1943). Two further male cases were reported and the condition was reviewed (Kitamura, Akamatsu & Hirokawa, 1953). The condition described by Kitamura corresponds most closely to the abnormalities seen in the present patients.

Clinical findings

Details of the patients in the present series are given in Table 1, and the clinical appearances are seen in Figs 1 and 2.

A network of sharply demarcated brown macules appeared on the extensor surfaces of the hands and feet in the 1st or 2nd decades of life. The lesions were irregularly angulated and freckle-like. They darkened gradually and extended proximally. In five cases, the lesions were more prominent in the summer.

The macules differed from ephelides in showing a retiform pattern and in being slightly depressed below the skin surface (Fig. 1, inset). Skin biopsies were taken from four of the patients (Cases 1, 3, 4 and 6). The limits of the macule were marked on the skin surface with Indian ink prior to the biopsy (Fig. 3, arrows). The epidermis between the markers was slightly atrophic. An increase in the number of clear cells in the basal region was seen in the haematoxylin and eosin-stained sections (Fig. 4). A DOPA oxidase stain revealed an excess of DOPA positive cells in the basal region (Fig. 5). There was no evidence of pigmentary incontinence in the upper dermis. All seven cases were familial,

Present address: University Department of Dermatology, Liverpool Royal Infirmary, Pembroke Place, Liverpool L3 5PU.

TABLE 1. Reticulate acropigmentation

Case	Sex	Age	Age at onset	Nationality	Ethnic group	Sites	Atrophy	Palmar pits	Other lesions	Family history
1. (K.Z.)	F	41	15	Iranian	Aryan	Arms Legs ~~Neck~~ Lower face Upper trunk	+	+	Plantar keratoderma	Mother of Case 2 7 others in family affected ~~but not seen~~
2. (S.Z.)	F	11	9	Iranian	Aryan	Arms Legs Neck Eyelids	+	–	—	Daughter of Case 1
3. (K.B.)	F	23	0–5	Iranian	Semitic	Arms Legs Neck Upper trunk	–	–	—	Mother and one sister affected
4. (S.W.)	F	26	0–5	British	Aryan	Arms Legs Neck	+	+	Psoriasis	Mother and maternal Grandmother also affected
5. (M.F.)	F	41	5–10	British	Aryan	Arms Legs Neck Face	–	+	Bilateral talipes	Mother of Case 4
6. (M.B.)	F	30	9	W. Indian	Negro	Arms Legs	+	+	Acne excoriée	Mother possibly affected but not seen
7. (G.T.)	F	30	?	Ghanian	Negro	Arms	+	+	Naevus spilus Naevus anaemicus Acrokeratosis elastoides	Mother also affected

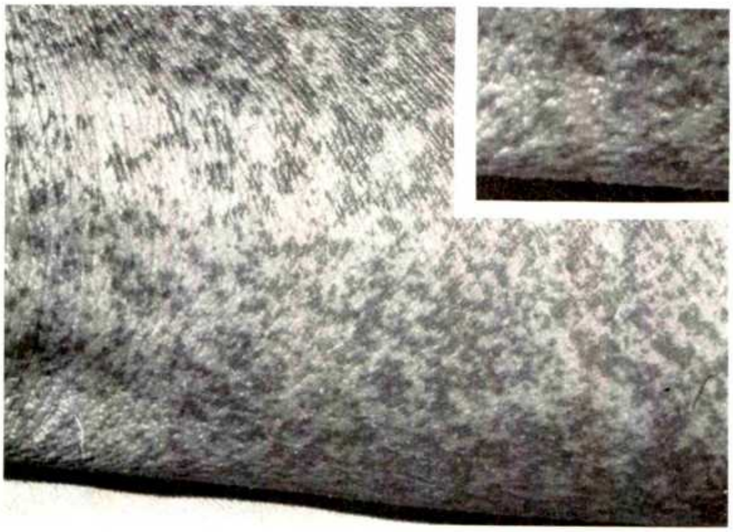


FIGURE 1. Freckle-like reticulate macules on arms (Case 1). Inset: close-up to show atrophy of the lesions

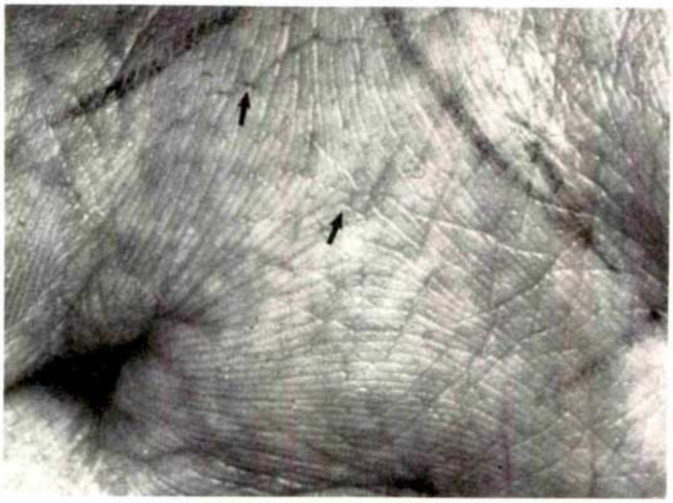


FIGURE 2. Close-up of palm to show numerous pits and breakages of epidermal ridge pattern (Case 1).

showing an autosomal dominant pattern of inheritance. In 5/7 cases, breaks in the epidermal ridge pattern of the palms and fingers were seen which extended over three or four ridges. Other genetically determined anomalies were noted in some of the present patients or their relatives: tylosis (Case 2), enamel hypoplasia (daughter of Case 4), bilateral talipes (Case 5), red hair, green eyes, pale skin and keratosis elastoides (paternal uncle of Case 7). All seven of the present patients were females.

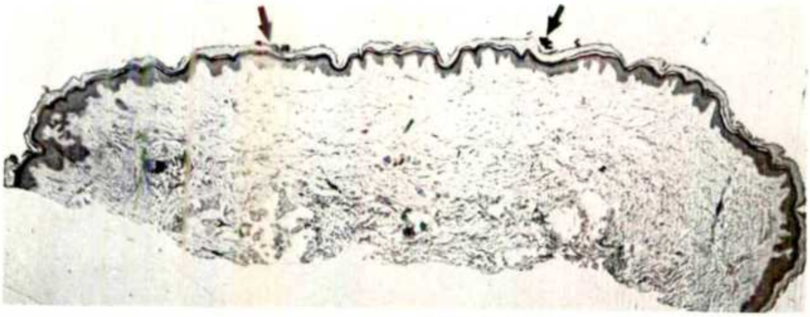


FIGURE 3. Whole biopsy The extent of the macule was marked on the surface with Indian ink (see arrows). Epidermal atrophy is seen between the arrows (H & E, × 25).

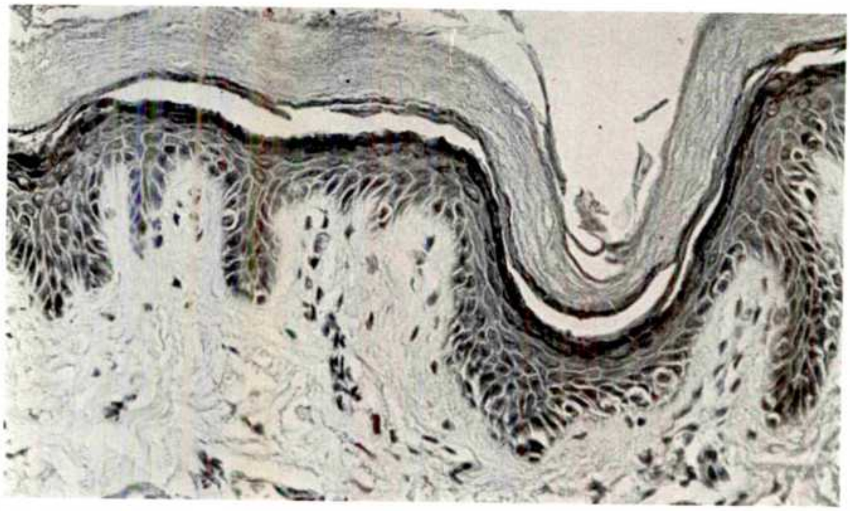


FIGURE 4. Detail of pigmented macule showing an excess of clear cells and epidermal atrophy (H & E, × 360).

DISCUSSION

The family reported by Becker & Reuter (1939) may have had the same disorder. They described three sisters, one aged 47 years and the other two twins aged 54 years, who had had pigmented macules on the chin, neck and arms for as long as they could remember. The lesions were brown, varied from 1 mm and 1 cm in diameter and were closely set, becoming confluent to form a pigmented network with normal-looking skin in between. A brother, aged 43 years, also had the anomaly. The father was living and well at the age of 91 years and had a similar pigmentary disorder. His sister died at the age of 86 years and also had the condition. The most prominent histological findings consisted of hypoplasia or atrophy of the stratum mucosum with an excess of melanin in the basal portion of the epidermis.

The differential diagnosis of Kitamura's reticulate acropigmentation includes a number of disorders which are thought to be genetically determined. The clinical features of the more important of these are listed in Table 2.

TABLE 2. Clinical features of some genetically determined pigmentary disorders

Disease	Distribution	Pattern	Hypo-pigmented lesions	Pigment incontinence	Comments
Acromelanosis	Acral	Diffuse	–	–	Negroid ethnic background
Acropigmentation of Kitamura	Acral	Reticulate	–	–	Palmar pits, breakage of epidermal ridges. Lesions atrophic
Acropigmentation of Dohi	Acral	Reticulate	+	–	Mixture of hyper- and hypo-pigmented macules. No atrophy. No palmar pits. Males predominantly affected
Franceschetti-Jadassohn Syndrome (Naegeli)	Generalized	Reticulate	–	+	Keratoderma, enamel hypoplasia, hypohidrosis
Dyskeratosis congenita	Generalized	Reticulate	±	+	Dystrophic nails & teeth, atrophy over extensor surfaces, occlusion of lachrymal duct, leukokeratosis, Fanconi anaemia. Sex-linked recessive
Dermatopathia pigmentosa reticularis	Generalized	Reticulate	+	+	Normal nails & teeth, no atrophy or leukokeratosis
Dyschromatosis universalis hereditaria	Generalized	Mottled	+	+	Mottled macules of differing shades & size, most prominent on trunk mixed with hypopigmented spots. Autosomal dominant.
Incontinentia pigmenti	Generalized	Whorls streaks	–	+	Usually females, may have early erythemato-bullous stage with later hyperkeratosis.
Familial progressive hyperpigmentation (Chernosky)	Generalized	Diffuse whorls streaks	–	–	No associated defects, dominant inheritance, no pigmentary incontinence.
Incontinentia pigmenti achromians (Ito)	Generalized	Whorls streaks	+	–	Hypopigmentation in reverse pattern of incontinentia pigmenti

Acromelanosis consists of diffuse hyperpigmentation of the fingers and shows no tendency to form a reticular pattern (Weidman, 1969).

Reticulate acropigmentation of Dohi is a familial anomaly, first described in ten Japanese males and two females under the title 'Symmetrical Pigment Anomaly of the Extremities' (Komaya, 1924). It differs from that described by Kitamura in showing a mixture of hyper- and hypo-pigmented macules, the absence of atrophy of the pigmented macules and of breaks in the epidermal ridge pattern. Komaya noted four different pigmentary patterns over the upper trunk and shoulders. The condition has also been recognized in Europeans (Gartmann, 1952; Siemens, 1964).

Generalized reticular pigmentation forms part of a number of genodermatoses. The Franceschetti–Jadassohn (Naegli) Syndrome has an earlier onset and is associated with keratoderma, enamel dysplasia and hypohidrosis (Franceschetti & Jadassohn, 1954).

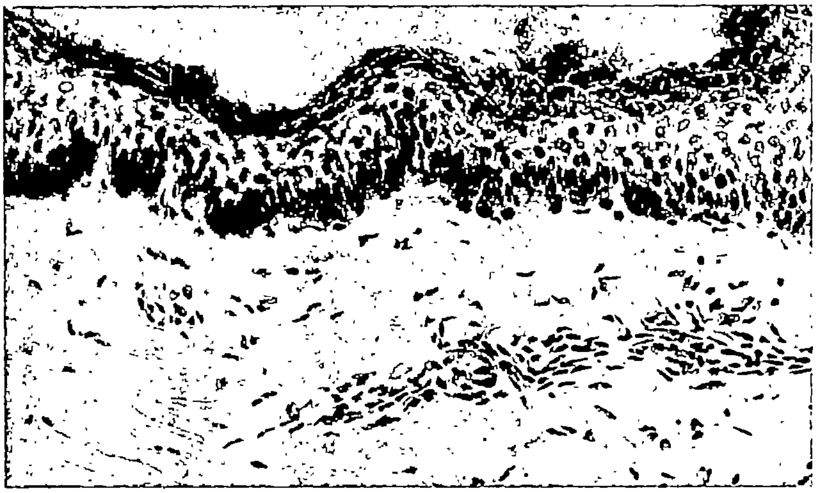


FIGURE 5. Detail of pigmented macule showing an excess of DOPA staining cells (DOPA oxidase ×360).

Dyskeratosis congenita almost always affects males and, in addition to generalized reticular pigmentation, shows nail atrophy, atrophy over the knuckles, elbows and knees, hyperkeratosis or occlusion of the lachrymal duct and leukoplakia of the oral mucosa. Some patients have developed internal adenocarcinomas. Aplastic anaemia of the Fanconi type occurs in some patients. The disorder is inherited as a sex-linked recessive (Jansen, 1951; Efron & Gallagher, 1971).

Dermatopathia pigmentosa reticularis (Hauss, 1958; Flegel, 1960; Rycroft, 1975) presents very similar cutaneous changes to dyskeratosis congenita but none of the cases so far reported has developed leukoplakia or internal malignancies. The four patients reported have all been females.

A generalized pigmentary disorder consisting of mottled pigmented macules of differing intensity intermixed with hypo-pigmented macules has been described under various titles including dyschromatosis universalis hereditaria (Ichikawa & Hiraga, 1933), leukoderma reticulare (Dohi, 1924) and generalized naevoid pigmentation (Wilson Jones, 1958).

Incontinentia pigmenti can readily be distinguished by the pattern of streaks and whorls and splashes of hyperpigmentation and by prominent pigmentary incontinence in the upper dermis.

Incontinentia pigmenti achromians (Ito) shows white streaks, whorls and splashes on a darker background (Griffiths & Payne, 1975).

Familial progressive hyperpigmentation (Chernosky *et al.*, 1971) is present at birth, or soon after, and shows spots, streaks and whorls of hyperpigmentation in all areas including the ocular and oral mucosae. Histologically, there is an increase in the number of basal pigment granules which were abnormal on electron microscopical examination. It differs from incontinentia pigmenti in lacking erythematous, vesicular and/or hyperkeratotic stages, and in the absence of histological evidence of pigment incontinence.

REFERENCES

BECKER, S.W. & REUTER, M.J. (1939) A familial pigmentary anomaly. *Archives of Dermatology*, 44, 987.

CHERNOSKY, M.E., ANDERSON, D.E., CHANG, J.P., SHAW, M.W. & RAMSDAHL, M.M. (1971) Familial progressive hyperpigmentation. *Archives of Dermatology*, 103, 581.

DOHI, S. (1924) Unusual pigment atrophy—leucoderma reticulata. *Japanese Journal of Dermatology*, **29**, 130.

EFRON, M.L. & GALLAGHER, W.F. (1971) Dyskeratosis congenita. in: *Dermatology and General Medicine*, (Ed. by T.B.Fitzpatrick *et al.*), p. 1109. McGraw Hill, New York.

FLEGEL, H. (1960) Dermatopathia pigmentosa reticularis. *Hautarzt*, **11**, 262.

FRANCESCHETTI, A. & JADASSOHN, W. (1954) A propos de 'L'incontinentia pigmenti', delimitation de deux syndromes differents figurant sous le meme terme. *Dermatologica*, **100**, 1.

GARTMANN, H. (1952) Akropigmentation symmetrica Dohi mit angeborenen Fundusveranderungen bei Mutter und Tochter. *Dermatologische Wochenschrift*, **125**, 534.

GRIFFITHS, A. & PAYNE, C. (1975) Incontinentia pigmenti achromians. *Archives of Dermatology*, **111**, 751.

HAUSS, H. (1958) Dermatopathia pigmentosa reticularis (Oberste-Lehn-Hauss). *Dermatologische Wochenschrift*, **49**, 1337.

ICHIKAWA, T. & HIRAGA, Y. (1933) Über eine noch nicht beschriebene Pigmentanomalie, Dyschromatosis universalis hereditaria Toyama, Ichikawa u. Hiraga. *Japanese Journal of Dermatology and Urology*, **34**, 66.

JANSEN, L.H. (1951) The so-called 'Dyskeratosis congenita'. *Dermatologica*, **103**, 167.

KITAMURA, K. & AKAMATSU, S. (1943) *Rinsho-no-Hifu-Hitsunyo*. **8**, 201.

KITAMURA, K., AKAMATSU, S. & HIROKAWA, K. (1953) Eine besondere Form der Akropigmentation: Acropigmentatio reticularis. *Hautarzt*, **4**, 152.

KOMAYA, G. (1924) Symmetrische Pigmentanomalie der Extremitaten. *Archiv für Dermatologie und Syphilis*, **147**, 389.

RYCROFT, R.J.G. (1975) Dermatopathia pigmentosa reticularis. *Proceedings of the Royal Society of Medicine*, **68**, 652 (title only).

SIEMENS H.W. (1964) Acromelanosis albo-punctata. *Dermatologica*, **128**, 86.

WEIDMAN, A.I. (1969) Acropigmentation (Acromelanosis). *Cutis*, **5**, 1119.

WILSON JONES, E. (1958) Generalized naevoid pigmentation. *Proceedings of the Royal Society of Medicine*, **51**, 710.

British Journal of Dermatology (1976) **95**, 445.

Pemphigus induced by rifampicin

R.W.GANGE,* E.L.RHODES,* C.O.EDWARDS† AND M.E.A.POWELL‡

Departments of *Dermatology, †Medicine, and ‡Pathology,
Kingston Hospital, Kingston upon Thames, Surrey

Accepted for publication 20 March 1976

We wish to report a case of pemphigus arising during treatment with rifampicin in which complete remission has taken place following withdrawal of the drug.

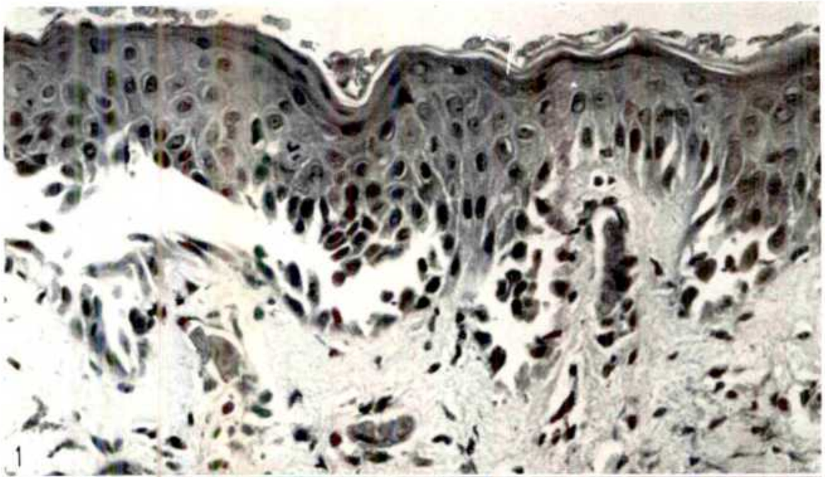
CASE REPORT

O.H., a woman of 65 years, had a nephrectomy in October 1973 for a non-functioning kidney. Histology indicated tuberculosis and treatment with rifampicin 300 mg twice daily, isoniazid 150 mg twice daily and ethambutol 700 mg twice daily was begun.

In July 1974 she was seen with a 2-week history of a blistering eruption over the trunk and limbs and eroded areas in her mouth. The bullae were both ruptured and intact and were surrounded by areas of erythema. She was admitted to hospital.

Biopsy of an early lesion showed suprabasal bulla formation and acantholysis (Fig. 1) and direct immunofluorescence showed intercellular IgG deposits throughout the depth of the epidermis (Fig. 2). Circulating antibodies to intercellular substance were present in the patient's serum to a titre of 1 in 5120.

Following admission her liver function tests became abnormal with raised transaminases and alkaline phosphatase (Fig. 3). A liver biopsy was performed which showed lymphocytic infiltration of the portal tracts but no evidence of liver cell damage. These changes were felt to be most likely due to rifampicin which was discontinued on 21.8.74, the ethambutol and isoniazid being given until October 1974. Circulating antibodies to rifampicin were not detected in her serum. The patient was treated with prednisone initially in a dosage of 15 mg three times a day and her skin eruption responded rapidly, the dosage being reduced to 10 mg twice daily on discharge. Her liver function tests showed rapid initial improvement and then continued to settle more slowly after leaving the hospital. Over the next 13 months her corticosteroid dosage was reduced gradually and finally discontinued in September 1975, fourteen months after presentation. At no time did she suffer any recurrence of the eruption and the titre of the antibody to intercellular substance gradually fell to 1 in 10.



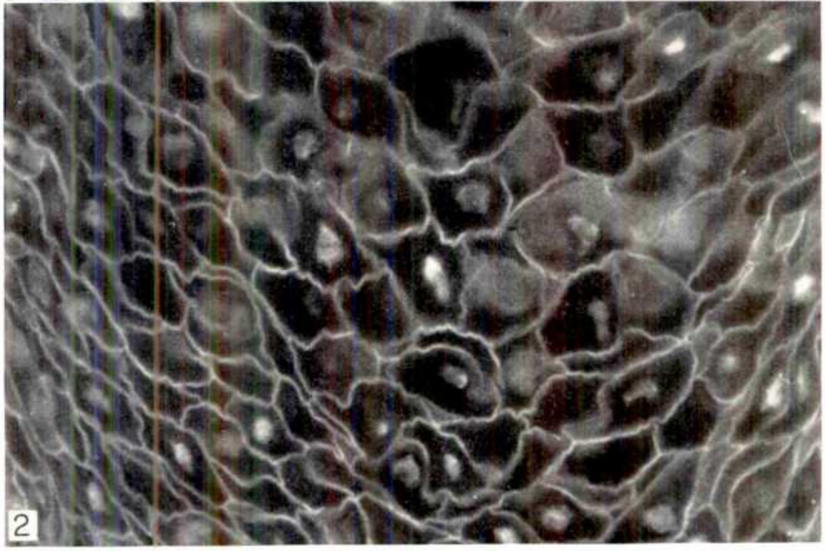


FIGURE 1. Biopsy showing suprabasal bulla formation and acantholysis.

FIGURE 2. Direct immunofluorescence showing intercellular IgG deposits throughout the depth of the epidermis.

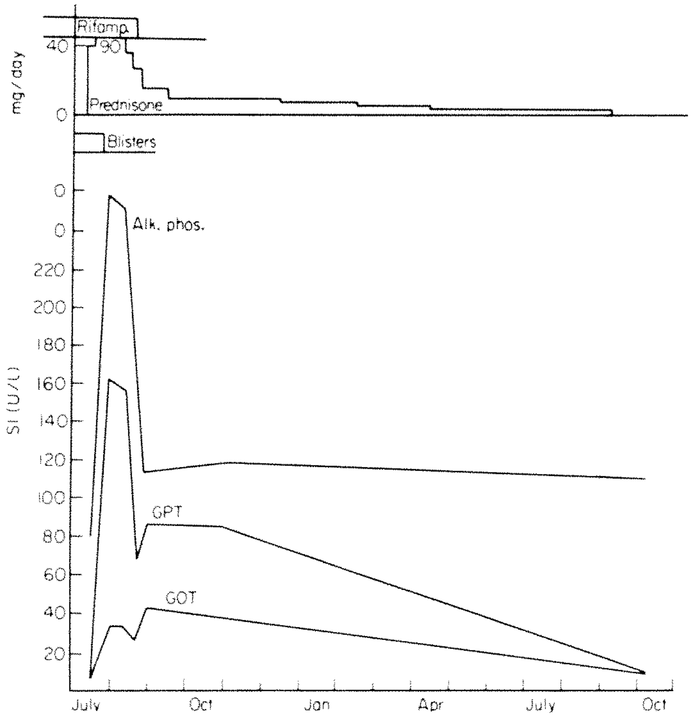


FIGURE 3. Biochemical changes during and after admission.

DISCUSSION

Drug induced pemphigus has been well recognized in recent years. Most reported cases have been related to penicillamine treatment (Degos *et al.*, 1969; Hewitt *et al.*, 1971, 1975) but other drugs such as phenylbutazone and irgapyrin have also been held responsible (Beutner, Chorzelski & Jordan, 1970). The clinical appearances are often atypical and more often resemble pemphigus foliaceus rather than pemphigus vulgaris. Of the nine cases reviewed by Hewitt *et al.* (1975), two had features of pemphigus vulgaris, six were 'seborrhoeic' in pattern and one had a 'pseudo-dermatitis herpetiformis' morphology. In some cases the severity of the eruption paralleled the dosage of the offending drug but did not always clear up on withdrawal (Degos *et al.*, 1969).

Some skin eruptions bearing no resemblance to pemphigus clinically have been found to be associated with pemphigus-like antibodies to the intercellular substance, such as those following the use of practolol (Fellner & Protkin, 1970; Amos, Brigden & McKerron, 1975). Electronmicroscopic studies revealed, in some cases, a pemphigus-like picture with widened intercellular spaces. Stein *et al.* (1972) reported deposition of immunoglobulin in the intercellular spaces in the epidermis in rashes caused by penicillin and chlorpromazine and a similar picture was found by Shelley, Schlappner & Heiss (1972) in a case of fixed drug eruption due to phenolphthalein. In these cases the staining was largely confined to the basal layer and no circulating antibodies were detected.

Rifampicin may cause skin reactions (Aquinas, 1973); when these occur they most commonly consist of flushing and itchiness of the face and scalp sometimes associated with a rash. Purpura may occur less commonly, either alone or with another rash. No pemphigus-like rashes have previously been described.

Liver function test abnormalities are well known in patients treated with rifampicin. In the case we are reporting pemphigus and liver abnormalities became apparent together about 10 months after treatment was started. Following withdrawal of the drug and treatment with corticosteroids, both conditions settled, the latter eventually being withdrawn completely and the pemphigus clearing. Antibodies to intercellular substance also faded from the serum.

While such clinical and serological remission may very occasionally occur in pemphigus, we feel that our case was drug induced because the pemphigus began at the same time as the liver enzymes became abnormal and both abnormalities disappeared following rifampicin withdrawal. Although this took a year in the case of the pemphigus, this was easily controlled with small doses of corticosteroids which were withdrawn a year later without recurrence of the rash.

REFERENCES

AMOS, H.E., BRIGDEN, W.D. & MCKERRON, R.A. (1975) Untoward effects associated with practolol. Demonstration of antibody binding to epithelial tissue. *British Medical Journal*, **i**, 598.

AQUINAS, M. (1973) Adverse reactions to daily and intermittent rifampicin and their management. *Ciba Symposium*, **2973**, 75.

BEUTNER, E.H., CHORZELSKI, T.P. & JORDAN, R.E. (1970) *Autosensitisation in Pemphigus and Bullous Pemphigoid*, p. 123. Charles C. Thomas, Springfield, Illinois.

DEGOS, R., TOURAINE, R., BELAICH, S. & REVUZ, J. (1969) Pemphigus chez un malade traité par penicillamine pour maladie de Wilson. *Bulletin de la société française de dermatologie et de syphiligraphie*, **76**, 751.

FELLNER, M.J. & PROTKIN, L.J. (1970) Morbilliform eruption caused by penicillin. A study by electron microscope and immunologic tests. *Journal of Investigative Dermatology*, **55**, 390.

HEWITT, J., LESSANA LEIBOWITCH, M., BENVENISTE, M. & SAPORTA, L. (1971) Un cas de pemphigus induit par la D-penicillamine. Le pemphigus iatrogène existe-t-il? *Medecine internationale*, **122**, 1003.

HEWITT, J., BENVENISTE, M. & LESSANA LEIBOWITCH, M. (1975) Pemphigus induced by penicillamine, an experimental pemphigus. *British Journal of Dermatology*, **93**, Supplement **11**, 12.

SHELLEY, W.B., SCHLAPPNER, O.L.A. & HEISS, H.B. (1972) Demonstration of intercellular immunofluorescence and epidermal hysteresis in bullous fixed drug eruptions due to phenolphthalein. *British Journal of Dermatology*, **86**, 118.

STEIN, K.M., SCHLAPPNER, O.L.A., HEATON, C.L. & DECHERD, W. (1972) Demonstration of basal cell immunofluorescence in drug induced toxic epidermal necrolysis. *British Journal of Dermatology*, **86**, 246.

British Journal of Dermatology (1976) **95**, 449.

Comment

CHARACTERISTICS AND CLINICAL IMPORTANCE OF KILLER (K)-CELLS

Cytotoxic or type II reactions (according to Coombs & Gell, 1975) are caused by antibody which is linked to an antigenic determinant on the cell surface, with participation of complement or of certain kinds of normal non-immune leukocytes. The complement-dependent reactions causing cell or tissue damage are well known phenomena as seen in ABO in incompatible transfusion reactions, drug reactions and others. In contrast, the cytotoxicity mediated by antibody and non-sensitized effector cells, therefore called antibody-dependent cellular cytotoxicity (ADCC), is complement-independent, demonstrable *in vitro* only, and its *in vivo* significance is more a matter of speculation; but there is evidence that effective defence mechanisms in the immune response to tumours could be stimulated in this way or possible links established in the pathogenesis of autoimmune or other diseases (Greenberg, Shen & Roitt, 1974).

The cells active in the ADCC-model are mainly called killer- or K-cells (MacLennan, 1974). Since E. Möller's report in 1965 many investigators have tried to clarify the nature of and mode of killing by these cells. Different target cells like chicken, sheep and human erythrocytes or cultured cells have been used together with antibodies from different species. It may be that these different conditions are the reason for the present differences of opinion about the nature of the cells and that indeed two or more populations of effector cells act in the ADCC-system.

Effector cells in the ADCC model possess receptors for the Fc-part of the IgG antibody which is bound to the surface of the target cell. Small amounts of IgG antibodies are sufficient to sensitize the target cell and cause adherence to the effector cells, and to trigger the cytotoxicity which is thought to be an extracellular killing and not a phagocytic mechanism (Perlmann, Perlmann & Wigzell, 1972; MacLennan, 1972). Using antibody-coated chicken erythrocytes as targets, monocytes, polymorphs and lymphocytes could be shown to be active in man, whereas antibody-coated tissue culture cells were lysed by lymphoid cells only (Perlmann *et al.*, 1975; MacDonald *et al.*, 1975). Therefore a heterogeneity of effector cells in the human peripheral blood can be demonstrated under appropriate experimental conditions. In connection with these results two questions have to be answered:

(1) Should all effector cells be called K-cells whatever the target cell used?

(2) What is the nature of the lymphoid effector cell?

The type of target cell is an important factor in ADCC. Monocytes, macrophages and polymorphs are not effective against antibody-coated tissue culture cells which resemble target cells *in vivo*. Differences in surface receptors should be included in any classification. The lymphoid effector cells seem to have no C3b-receptor (Ramshaw & Parish, 1976; De E. de Bracco *et al.*, 1976; Kraft *et al.*, 1976; Eremin *et al.*, 1976b) unlike monocytes, macrophages and polymorphs which have. Furthermore, inhibition experiments showed that the Fc-receptors of human blood monocytes are different from those on the lymphoid cells displaying ADCC-activity. Taken together, these data suggest there is a lymphoid effector cell which is the real K-cell (Perlmann *et al.*, 1975) and other cells which are well characterized as monocytes, macrophages and polymorphs and do not need further characterization.

The exact nature of the lymphoid effector cells remains a controversial issue. In rodents the inability of neonatal thymectomy or of treatment with anti T-lymphocyte antiserum and complement to affect the cytotoxic cells in ADCC make it unlikely that T-cells are involved (Greenberg, Shen & Roitt, 1974). In man various authors have independently shown that lymphocyte preparations depleted of T-cells demonstrate enhanced ADCC-activity (Wislöff *et al.*, 1974; Perlmann *et al.*, 1975). Evidence for B-cells as effector cells has been presented (MacLennan, 1972; Chess *et al.*, 1974) but contradicted by others (Wislöff & Frøland, 1973). At the moment studies support the existence of a lymphocyte subpopulation between T and B-cells (Wislöff *et al.*, 1974) which lacks both the capacity to form rosettes with sheep erythrocytes and the presence of surface immunoglobulin (null cells according to MacDermott *et al.*, 1975). Whether these cells are precursors of either the T or the B lymphocyte series or a distinct cell line equivalent to the non-adherent non-phagocytic mouse K-cell (Greenberg *et al.*, 1975) is unknown. But the possibility exists that there could be heterogeneity within this population.

Reports from several laboratories suggest that destruction of antibody-coated target cells by effector cells may have an important role in human host defence mechanisms. Functionally, the extracellular cytotoxicity is expected to be effective where the target cell is too large for ingestion by phagocytosis, e.g. large parasites and solid tumours. It was shown *in vitro* that targets like Cryptococcus neoformans (Diamond, 1974), schistosumola mansoni (Butterworth, Sturrock & Rees, 1974), culture forms of Trypansoma dionisii (Mkwananzi *et al.*, 1976) or Herpes simplex 1-infected culture cells (Shore *et al.*, 1974) could be destroyed by normal human blood cells after coating with the specific antibody. It would be premature to claim that these results establish a role for ADCC in immunity against fungal, protozoal and viral infections. Nevertheless, this type of cytotoxicity could be a factor limiting the spread of some infectious agents from one cell to another.

In the immune response to tumours, circulating antibodies have been found which could be cytotoxic for isolated tumour cells. To destroy solid tumours cytotoxic T and K-cells seem to be needed. Studies in patients with tumours, for example malignant melanoma (Peter, Knoop & Kalden, 1976) and breast carcinoma (Eremin, Ashby & Franks, 1976a), showed a reduced ADCC-activity of peripheral blood lymphocytes compared with that of normal persons. These results could be better explained by a reduction of K-cells than by 'blocking factors'. On the other hand sera from patients with transitional cell carcinoma of the urinary tract could confer tumour-specific cytotoxicity upon lymphocytes from normal donors (Hakala *et al.*, 1974). Therefore, sera from tumour patients seem to contain cytophilic antibody or antigen–antibody complexes with free antibody valencies which can arm lymphocytes and allow the interaction of 'armed' effector cells with the target-antigen to trigger ADCC. In this way the cytotoxicity could be enhanced and may correlate with decreased tumour burden. But it is important to bear in mind that under certain circumstances soluble immune complexes are known to inhibit the effector cells of ADCC and could in fact protect the tumour from cellular attack (Hellström & Hellström, 1974). A similar blocking of ADCC was seen in SLE and rheumatoid arthritis and may be related to DNA-anti-DNA complexes and aggregated IgG respectively (Scheinberg & Cathcart, 1976).

Other suggested K-cell activities include rejection of kidney transplants (D'Apice & Morris, 1974; Ting & Terasaki, 1974), but investigations are needed to draw firm conclusions.

In summary, K-cells are characterized as effector cells mediating antibody-dependent cellular cytotoxicity *in vitro* but their relevance to immunological defence mechanisms *in vivo* (resistance to infectious diseases, tumour regression, allograft rejection) remains to be established.

Institute of General and Experimental Pathology,
University of Vienna,
Währingerstrasse 13,
A-1090 Vienna, Austria

DIETRICH KRAFT

REFERENCES

Butterworth, A.E., Sturrock, R.F. & Rees, P.H. (1974) Antibody-dependent cell mediated damage to schistosomula *in vitro*. *Nature*, **252**, 503.

Chess, L., MacDermott, R.P., Sondel, P.M. & Schlossmann, S.F. (1974) Isolation and characterization of cells involved in human cellular hypersensitivity. *Progress in Immunology*, **11**, 125.

Coombs, R.R.A. & Gell, P.G.H. (1975) Classification of allergic reactions responsible for clinical hypersensitivity and disease. In: *Clinical Aspects of Immunology* (Ed. by P.G.H. Gell, R.R.A. Coombs and P.J. Lachmann), 3rd edn, p. 761. Blackwell Scientific Publications, Oxford.

D'Apice, A.J.F. & Morris, P.J. (1974) The role of antibody-dependent cell mediated cytotoxicity in renal allograft rejection. *Transplantation*, **18**, 20.

De E. de Bracco, M.M., Isturiz, M.A. & Manni, J.A. (1976) Cell-mediated cytotoxicity. Characterization of the effector cells. *Immunology*, **30**, 325.

Diamond, R.D. (1974) Antibody-dependent killing of Cryptococcus neoformans by human peripheral blood mononuclear cells. *Nature*, **247**, 148.

Eremin, O., Ashby, J. & Franks, D. (1976a) Killer cell (K) activity in human normal lymph node, regional tumour lymph node and inflammatory lymph node. *International Archives of Allergy and Applied Immunology*, in press.

Eremin, O., Kraft, D., Franks, D., Coombs, R.R.A., Ashby, J. & Plumb, D. (1976b) Characterization of the human killer (K) cell: a lymphocyte with an Fc receptor but lacking a C′3 receptor on its surface membrane *International Archives of Allergy and Applied Immunology*, submitted for publication.

Greenberg, A.H., Shen, L. & Roitt, I.M. (1974) Characteristics of the effector cells mediating antibody-dependent cytotoxicity. *Current Titles in Immunology, Transplantation and Allergy*, **1**, 33.

Greenberg, A.H., Shen, L., Walker, L., Arnaiz-Villena, A. & Roitt, I.M. (1975) Characteristics of the effector cells mediating cytotoxicity against antibody-coated target cells. II. The mouse non-adherent K cell. *European Journal of Immunology*, **5**, 475.

Hakala, Th. R., Lange, P.H., Castro, A.E., Elliott, A.Y. & Fraley, E.E. (1974) Antibody induction of lymphocyte-mediated cytotoxicity against human transitional-cell carcinomas of the urinary tract. *Nev England Journal of Medicine*, **291**, 637.

Hellström, K.E. & Hellström, I. (1974) Lymphocyte mediated cytotoxicity and serum blocking activity to tumor antigens. *Advances in Immunology*, **18**, 209.

Kraft, D., Franks, D. & Coombs, R.R.A. (1976) Characterization of K-cell activity by use of depletion experiments. *International Archives of Allergy and Applied Immunology*, in press.

MacDermott, R.P., Chess, L. & Schlossmann, St.F. (1975) Immunologic functions of isolated human lymphocyte subpopulations. V. Isolation and functional analysis of a surface Ig negative, E rosette negative subset. *Clinical Immunology and Immunopathology*, **4**, 415.

MacDonald, H.R., Bonnard, G.D., Sordat, B. & Zawodnik, S.A. (1975) Antibody-dependent cell-mediated cytotoxicity: heterogeneity of effector cells in human peripheral blood. *Scandinavian Journal of Immunology*, **4**, 487.

MacLennan, I.C.M. (1972) Antibody in the nduction and inhibition of lymphocyte cytotoxicity. *Transplantation Reviews*, **13**, 67.

MacLennan, I.C.M. (1974) Comments on antibody-dependent cell (K) mediated cytotoxicity. In: *Advances in the Biosciences 12, Schering Symposium on Immunopathology*, p. 87. Pergamon Press, Oxford.

Mkwananzi, J.B., Franks, D. & Baker, J.R. (1976) Cytotoxicity of antibody-coated trypanosomes by normal human lymphoid cells. *Nature*, **259**, 403.

Möller, E. (1965) Contact-induced cytotoxicity by lymphoid cells containing foreign isoantigens. *Science*, **147**, 873.

Perlmann, P., Perlmann, H. & Wigzell, H. (1972) Lymphocyte mediated cytotoxicity *in vitro*. Induction and inhibition by humoral antibody and nature of effector cells. *Transplantation Reviews*, **13**, 91.

Perlmann, P., Perlmann, H., Larsson, A. & Wahlin, B. (1975) Antibody dependent cytotoxic effector lymphocytes (K cells) in human blood. *Journal of the Reticuloendothelial Society*, **17**, 241.

Peter, H.H., Knoop, F. & Kalden, J.R. (1976) Spontaneous and antibody-dependent cellular cytotoxicity in melanoma patients and healthy control persons. *Zeitschrift für Immunitätsforschung*, **151**, 263.

Ramshaw, I.A. & Parish, C.R. (1976) Surface properties of cells involved in antibody-dependent cytotoxicity. *Cellular Immunology*, **21**, 226.

Scheinberg, M.A. & Cathcart, E.S. (1976) Antibody-dependent direct cytotoxicity of human lymphocytes.

I. Studies on peripheral blood lymphocytes and sera of patients with systemic lupus erythematodes. *Clinical and Experimental Immunology*, **24**, 317.

SCHEINBERG, M.A. & CATHCART, E.S. (1976) Antibody-dependent direct cytotoxicity of human lymphocytes. II. Studies on peripheral blood lymphocytes, synovial fluid cells and sera from patients with rheumatoid arthritis. *Clinical and Experimental Immunology*, **24**, 323.

SHORE, S.L., NAHMIAS, A.J., STARR, S.E., WOOD, P.A. & MCFARLIN, D.E. (1974) Detection of cell-dependent cytotoxic antibody to cells infected with herpes simplex virus. *Nature*, **251**, 350.

TING, A. & TERASAKI, P.I. (1974) Influence of lymphocyte-dependent antibodies of human kidney transplants. *Transplantation*, **18**, 371.

WISLÖFF, F. & FRÖLAND, S.S. (1973) Antibody-dependent lymphocyte mediated cytotoxicity in man: no requirement for lymphocytes with membrane-bound immunoglobulin. *Scandinavian Journal of Immunology*, **2**, 151.

WISLÖFF, F., FRÖLAND, S.S. & MIHAELSEN, T.E. (1974) Antibody dependent cytotoxicity mediated by human Fc-receptor bearing cells lacking markers for B and T lymphocytes. *International Archives of Allergy and Applied Immunology*, **47**, 139.

British Journal of Dermatology (1976) 95, 453.

Thesis

The differentiation of dermatophytes by biochemical and serological methods. CHRISTINE M.PHILPOT (1976) University of London.

In the past, classification of dermatophyte fungi depended on morphological criteria with the result that many species have been erected on minor differences in structure or on isolation from an unusual host or from a particular clinical condition. This pattern is slowly changing as biochemical techniques, which have been applied so successfully to other groups of micro-organisms, are applied to the fungi.

Ability to hydrolyse urea allows differentiation of *Trichophyton mentagrophytes* from *T. rubrum* and *T. erinacei* and of *T. soudanense* from *M. ferrugineum.* Although the overall similarity of the nutritional patterns of 22 dermatophyte species on 30 carbohydrate or nitrogen sources precluded any definitive scheme, the results supported classifications based on morphology. In particular the segregation of *Microsporum equinum* as a separate species receives support from nutritional studies. Serological investigations were carried out in tandem with the nutritional studies.

Sonically-disrupted, formalin-killed mycelium was used to inoculate rabbits. Precipitating antibodies in the serum were sought by double diffusion techniques in agar gel. Relationships between strains were based on the presence of common antigens. Most current members of the genus *Microsporum* were distinct from the genus *Trichophyton*; exceptions were *M. gypseum* and *M. persicolor* which shared antigens with *T. ajelloi.* Two distinct sub-groups were seen in *Trichophyton*; one contained *T. mentagrophytes*, *T. rubrum* and *T. schoenleinii* and the other *T. quinckeanum*, *T. soudanense* and *T. erinacei.* Both *T. violaceum* and *Epidermophyton floccosum* stood apart from the other groups as did two saprophytic species *T. terrestre* and *M. cookei.*

It is hoped that this thesis heralds the start of a new and closer look at the taxonomy of dermatophyte fungi.

W.C.NOBLE

The role of bacteria in acne vulgaris. C.D.ROBERTS (1976) University of Leeds.

Using a standardized glass sampling rod and cyano-acrylate glue a study was made of organisms found in the pilosebaceous duct of persons with or without acne vulgaris. *Corynebacterium acnes* was found to inhabit the ducts whilst members of the Micrococcaceae were apparently confined to the upper layers of the skin. No difference was found between quantitative counts of organisms on normal skin compared with apparently normal skin of patients with clinical acne. However counts were significantly lower in areas of acne; this was so for the *C. acnes* complex as well as Micrococcaceae. The capacity of these organisms to produce lipase, protease and hyaluronidase *in vitro* was not related to the area of skin from which they were isolated.

Continuous and batch cultures of *C. acnes* in a chemostat showed that *C. acnes* could utilize oxygen and this reinforces suggestions that it be included in the genus *Propionibacterium.* Oxygen tension and pH were found to have marked effects on growth rate of the organism and on production and yield of extracellular enzymes. Growth rate optima were pH 6·0 and 40 mmHg; maximum yield occurred at pH 5·3 and 60 mmHg. Enzyme production was optimal for lipase at pH 5·75, 40 mmHg; but for protease at pH 6·5, 0 mmHg and hyaluronidase pH 5·75, 0 mmHg oxygen tension.

Clearly small changes in oxygen tension and pH could lead to marked changes in enzyme production if such conditions also prevail *in vitro.* Lesion production cannot apparently be attributed to changes in microbial numbers *in vivo* but may relate to comparatively small changes in growth conditions of the organism *in situ.* However the conditions under which the organism grows *in vitro* remain to be determined.

W.C.NOBLE

Book Reviews

Dermatology. S.L.MOSCHELLA, D.M.PILLSBURY and H.J.HURLEY (1975) Philadelphia: W.B. Saunders. Pp. 1751. Price £45.

Dermatology by Pillsbury, Shelley and Kligman (1956) was rightly one of the most successful textbooks of 20 years ago. This 1975, two volume *Dermatology* is not meant to be a second edition so much as an enlarged successor. There are now 43 authors. It is interesting to find how aptly the sections on diagnosis and on eczema can be virtually reprinted from 20 years ago, including many of the original very fine clinical photographs and diagrams now shown to even better advantage. The other sections have been entirely reshaped, rewritten and expanded to double the size of the 1956 *Dermatology*. There are well illustrated introductory chapters on anatomy and physiology, basic histological diagnosis and concluding chapters on radiation therapy and on skin diseases in black races.

In general the approach is orthodox and the problems of a multi-author book have been got over. There is not too much duplication, the book reads easily, and is attractively produced. The black and white illustrations are mainly good, but some are surprisingly disappointing.

Compared with other major textbooks of dermatology this one has marginally more illustrations and less text. The bibliography is very ample, with the advantages and disadvantages that the majority of the references are from the easily accessible English language literature. There is a suggestion from some of the chapters that the book has had a longish gestation.

The 1956 *Dermatology* had especially in mind the viewpoint of students and physicians with little or no experience with skin diseases. This book will appeal to the same groups, but is also a more major reference text for dermatologists. Although some eyebrows may be raised at another major textbook, there are excellent historical precedents for this work and the price is competitive.

R.H.CHAMPION

Dermatology. HANS RORSMAN (1976) Lund: Studentlitteratur, Wolfe Medical Books. Pp. 252.

It is always difficult to know how much dermatology to try and teach medical students. From a practical point of view it depends on the time available; this is usually all too short and allows no more than a smattering to be presented, so that the first problem facing the teacher is to decide what to leave out, and yet allow a coherent account of useful information to be given in a readable and memorable form. Within these limitations Professor Hans Rorsman has done a marvellous job. If such a book is to be clear, definite and concise, as this one is, it must inevitably be stylised, and dermatologists are bound to disagree on what appears, as well as what does not.

Perhaps the best way to judge the book would be to try and define how I think it would prepare a student for the battle of medical life. The author has emphasised that it must be used in conjunction with an Atlas of Dermatology, as well as with 'a lavish demonstration of patients.' These are essential provisos because, apart from the freckled red-head in the vignette on the cover, the book contains only a few line drawings, and it represents no more than the skeleton of the student's requirement, upon which the living flesh of clinical dermatology must be raised. Granted these conditions I think that he (or she) would have been well initiated, with a good sense of proportion. I appreciated the emphasis on things of practical importance such as contact dermatitis and syphilis. He would have been given an insight into the pathogenesis of cutaneous disease, the limitations of classification, the differential diagnosis of common eruptions, a definite line of treatment to follow, and advice about referral to specialists; but I must iterate that the key to unlock this information lies in the clinic and the atlas. He might be at somewhat of a disadvantage in those areas where internal medicine and dermatology meet, as witness the emptiness of the page headed *myxoedema*. On the other hand the usually neglected field of oral medicine is covered, if rather theoretically, for traumatic lesions are omitted and the importance of deficiencies of serum iron, B 12 (and folate) are related to glossitis only. (Incidentally sucking a peach stone is an excellent way of denuding a black hairy tongue.) He would be badly informed about topical steriod red face, reluctant to diagnose lichen simplex (except on the nape, in the ano-genital region, or on the male shin), and he would miss pruritus due to cholestasis unless the patient was jaundiced.

The student would not be burdened by useless information, and in fact it would be difficult to decide what to leave out if the book had to be shortened by, say, five pages. I would omit amyloidosis, rheumatoid nodules (including them under granuloma annulare), polyarteritis nodosa and the Gianotti–Crosti syndrome (what's that? said one of the staff), and would combine xanthoma and xanthelasma into one page and include tendon xanthoma. The choice depends on the geographical incidence of disease, a subject touched on in the introduction. The figures given for Lund would be more instructive if they could be compared with somewhere else — I am aware of the difficulties. I was baffled to know what the 46·8% of patients were suffering from who were not listed in the 21 commonest diagnoses. If the five freed pages were to be re-allotted I should like to see some or more information about mosaic plantar warts, orf, tinea incognito, cattle ringworm, scabies, pet animal infestations, lichen simplex and associations of internal disease with the skin. In this context the prescription of a blunderbuss application containing DDT, benzyl benzoate and tetramethylthiuram disulphide for scabies seems unwise, especially as mites are not sensitive to DDT. Some other points need modification. For example I would suggest that pityriasis rosea is often an itchy disease, that necrobiosis is not necessarily confined to the legs, and that the basis of artefacts is more likely to be emotional immaturity than psychosis. The characteristic way in which healing lichen planus pigments has been overlooked, and it might be better to refer to serological tests for syphilis (STS) rather than the WR. But the fact is that this is a good book for students provided that it is supplemented, as the author intends, by seeing many patients and looking at many pictures.

ALAN LYELL

Skin care. BETHEL SOLOMONS (1975) London: Priory Press. Pp. 151. Price £5.00.

This small book is an addition to the Care and Welfare Library. It has been written with the object of presenting to the layman the basic facts of the common, and occasionally less common, disorders of the skin. Although it is easy to fault these books, I doubt whether many of us could have done better. Bethel Solomons' rather colloquial style is well suited to a publication of this type, particularly when he is dealing with fringe topics, such as cosmetics. Occasionally the balance of information seems somewhat inappropriate: for example, in the section on the eye, I would have preferred a reference to xanthelasma to the description of nits of the lashes. Nevertheless, in the main the author has succeeded in presenting a very readable account of the central problems of our speciality. All Dermatologists should welcome books of this nature as an indication of increasing awareness of the importance of the sociological implications of skin disease. We need to know far more concerning what people believe and do about skin problems before they go to their Doctor, let alone a Dermatologist.

ERNEST FAIRBURN

Varizen—Ulcus cruris und Thrombose. KARL SIGG (1976) Berlin: Springer-Verlag. Pp. 403, colour and B/W illustrations. Price DM 168.

This, the 4th edition, commences with detailed anatomical and physiological descriptions of the circulation of the lower limb, followed by consideration of the various anomalies of the venous circulation and their effects on the nutrition of the tissues. Separate sections of the book deal at length with thrombophlebitis and venous ulceration while shorter ones cover lymphoedema, lipodistrophy and compression therapy. The various forms of surgical, sclerotherapy and medical treatments are discussed in detail. The work is profusely illustrated with both black and white as well as colour photographs — in places these are somewhat repetitive. There are also excellent line drawings and radiographs which add to the clarity of the text.

As well as drawing on his own vast experience, Sigg quotes the views of many authors, including some from the United Kingdom. The bibliography covers 21 pages of which 3 are devoted entirely to publications by Karl Sigg himself. Where mention has been made of proprietory materials, be they scalpel blades, sclerosants, topical steriods or bandages, full details are provided in an appendix, with the addresses of the manufacturers.

The work concludes with an accurate index. This is a valuable reference book for those surgeons, dermatologists and others who are interested in this important subject which is responsible for considerable morbidity. It deserves translation into the English language.

NAPIER THORNE

British Journal of Dermatology (1976) **95**, 457.

News and Notices

Investigative meeting of the British Association of Dermatologists, 21 & 22 January 1977

This meeting will take place on the afternoon of Friday, 21st and the whole of Saturday, 22nd January, 1977. It will take place at Guy's Hospital Medical School. Please could abstracts be forwarded to Dr W.J. Cunliffe, Consultant Dermatologist, Department of Dermatology, The General Infirmary, Great George Street, Leeds LS1 3EX, by not later than 29 October, 1976.

Five copies of the abstract should be typed double spaced on 8·5 × 11 in. or 21 × 29·5 cm paper. The abstract should contain a statement of the purpose of the study, methods used and a summary of the results presented in sufficient detail to support the conclusions. Supplementary information, such as electron micrographs and tables, can be submitted at the discretion of the author for the use of the programme committee.

It is hoped that Stiefel Laboratories Limited will find appropriate accommodation, at a reasonable price, in a hotel close to Guy's Hospital Medical School. There will be a subsidised meal on the evening of Friday, 21 January, 1977.

Full details of the meeting will be circularized to members of the BAD and of the Investigative Group later in the autumn. Others interested should contact Dr Cunliffe.

Symposium on cosmetic and pharmacological aspects of skin colour

The Pharmaceutical Society of Great Britain and the Society of Cosmetic Chemists of Great Britain will hold a Joint Symposium on Cosmetic and Pharmacological Aspects of Skin Colour at the Stratford-upon-Avon Hilton, Stratford-upon-Avon on 9–11 November, 1976.

For details and registration (before 11 October) contact the Society of Cosmetic Chemists of Great Britain, 56 Kingsway, London WC2B 6DX.

Erratum

The following references were omitted from Supplement 13 '*Toxic Woods*' (Vol. 94, June 1976):

ANON. (1951) *Specimen Woods No.* 191: Musine. Wood (supp.) **16**(11).

ANON. (1954) Mukulungu. *Bois et Forêts des Tropiques*, **36**, 25–28.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of the Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: CUNLIFFE, W.J. & SHUSTER, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: BEARE, J.M. & WILSON JONES, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066. Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 95, Number 4, October 1976

CONTENTS

Page

Clinical and Laboratory Investigations

Practical Applications of the Quantimet 720, an Image Analysing Computer, in the Field of Investigative Dermatology 341
W.PICTON, H.DEVITT AND M.A.FORGIE

The Lines of Blaschko: a Review and Reconsideration. Observations of the Cause of Certain Unusual Linear Conditions of the Skin 349
ROBERT JACKSON

The Effect of Collagenase on Surface Markers of Human Peripheral Lymphocytes 361
TULIP TAN

Infantile Eczema. A Long-term Follow-up Study 365
KEITH MUSGROVE AND JOHN K.MORGAN

Psychological Profile of Patients with Atopic Dermatitis 373
HANA F.AL-AHMAR AND AMAL K.KURBAN

Hypersensitivity to Bacteria in Eczema. III. Arthus-like Responses to Bacterial Antigens Without Specific Antibody 379
ESTHER WELBOURN, R.H.CHAMPION AND W.E.PARISH

Pigmented Lesions in Newborn Infants 389
ROBERT G.WALTON, ALVIN H.JACOBS AND ALVIN J.COX

The Effects of Photosensitizers and Ultraviolet Irradiation on the Biosynthesis and Metabolism of Prostaglandins 397
JONATHAN T.LORD, VINCENT A.ZIBOH, JOSEPH POITIER, GERALD LEGGET AND NEAL S.PENNEYS

Subcutaneous Fat Necrosis of the Newborn. Light and Electron Microscopic Studies 407
TAKUO TSUJI

Metal Corrosion by Chloride in Sweat. The Problem of 'Rusters' in Industry 417
J.L.BURTON, R.J.PYE AND D.H.BROOKES

Pharmacology and Treatment

Proprietary Hydrocortisone Creams. Vasoconstrictor Activities and Bio-availabilities of Six Preparations 423
B.W.BARRY AND R.WOODFORD

Effect of 1% Cyproterone Acetate in Cetomacrogol Cream BPC (Formula A) on Sebum Excretion Rate in Patients with Acne 427
R.J.PYE, J.L.BURTON AND J.I.HARRIS

Case Reports

Disseminated Superficial Actinic Porokeratosis. Analysis of an Affected Family 429
DONALD J.PIROZZI AND ALBERT ROSENTHAL

Unilateral Epidermal Naevus Resembling Acanthosis Nigricans 433
HELEN OLLENDORFF CURTH

Reticulate Acropigmentation of Kitamura 437
W.A.D.GRIFFITHS

Pemphigus Induced by Rifampicin 445
R.W.GANGE, E.L.RHODES, C.O.EDWARDS AND M.E.A.POWELL

Comment

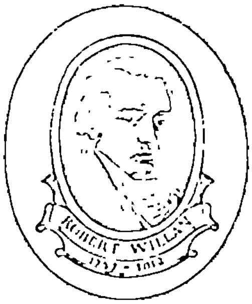
Characteristics and Clinical Importance of Killer (K)-cells 449
DIETRICH KRAFT

Ph.d Theses and Book Reviews 453

News and Notices 457

8.2.77

Volume 95, Number 5, November 1976



British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

British Journal of Dermatology

EDITOR

DR R. H. CHAMPION
Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

DR J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London
DR RONALD MARKS, Cardiff
DR P.D.MIER, Nijmegen
DR J.N.S.MITCHELL, Royal Air Force
DR W.C.NOBLE, London
DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS

DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 U.K. (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed in Great Britain at the Alden Press, Oxford

PRESCRIBE BY NAME

Salactol*

TRADE MARK

THE ESTABLISHED TREATMENT FOR WARTS

containing
Salicylic acid 16·7%
Lactic acid 16·7%
in a collodion base

Full prescribing information is available
*Salactol is a trade mark of Dermal Laboratories Ltd
Gosmore, Nr. Hitchin, Herts SG4 7QR

Basic N.H.S. Price 59p
Bulk discounts available
to hospitals

PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS

the modern basis of treatment

'. . . to restrict topical steroid therapy to short periods for getting the disease under control and then to change to tar preparations which may prevent rebound exacerbations.'

Practitioner, 1970, 204, 625.

New Alphosyl HC cream

for short-term control

The unique descaling, healing and anti-inflammatory properties of Alphosyl, plus the added anti-inflammatory action of hydrocortisone to get the disease swiftly under control.

Alphosyl cream

lotion and shampoo for long-term maintenance

'. . . it (Alphosyl) does not have the disadvantage of staining, burning and discolouration that occur with other topical preparations.' Br.med.J., 1967, 4, 535.

Alphosyl

a whole range of treatment for psoriasis

Alphosyl cream, lotion and shampoo contain a highly refined extract of coal tar and allantoin. Alphosyl HC in addition contains 0.5% hydrocortisone. Please send to us for samples, clinical information and Data Sheets.

Stafford-Miller The Professional Relations Division, Stafford-Miller Limited, Hatfield, Herts.

'OUTLOOK IN PSORIASIS'

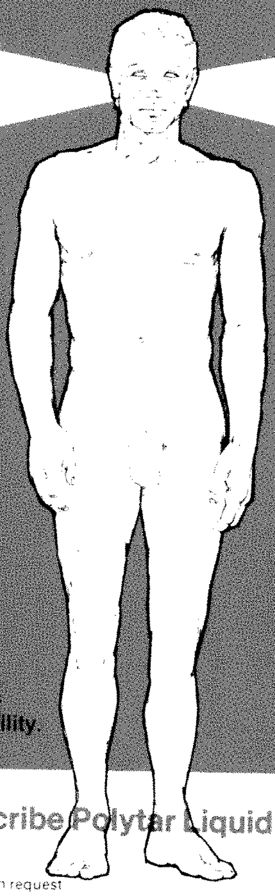
'The time-honoured applications of tar or dithranol and exposure to ultra-violet light remain the basis for local treatment'

Editorial, Brit. med. J. 1974,4,365

Polytar Liquid Psoriasis of the scalp

In a recent teaching hospital study, Polytar Liquid was compared with a standard cetrimide formulation in 50 patients with psoriasis of the scalp. Polytar Liquid proved superior in the removal of scale and the relief of oiliness. Patient acceptability was excellent.

Polytar Liquid combines effective control with patient acceptability



Polytar Emollient Psoriasis of the body

Polytar Emollient is a tar bath additive which does not stain the bath surface, hair, skin or nails. In a double blind controlled trial with 90 patients Polytar Emollient proved superior to liquor picis carb. in terms of patient and nursing staff acceptability.

"Polytar Emollient has a useful part to play in the treatment of psoriasis, particularly in out-patient treatment".

Clin. Trials J. 1970, 4, 438

Polytar Emollient superior to liquor picis carbonis

For psoriasis – prescribe Polytar Liquid and Polytar Emollient

Full information on request

Stiefel Laboratories (UK) Limited 825 Yeovil Road, Slough, SL1 4JA

IMPORTANT NEW PRODUCT FOR PSORIASIS

olan
Dithranol

Much easier to apply, far easier to remove, and actually more effective.

DITHROLAN is quite simply the most convenient and effective form of dithranol in the treatment of psoriasis.

A careful and painstaking study carried out over the last 5 years at the Beaumont Hospital, Lancaster, demonstrated that a newly developed formulation of dithranol, now introduced as DITHROLAN, was not only very much easier to use than traditional Dithranol Paste, but was actually more effective[1, 2].

DITHROLAN adheres easily to the skin and can be applied quickly and conveniently. Previous applications of DITHROLAN can be removed simply by bathing – obviating the need for oil. As a result, patients actually carry out their treatment.

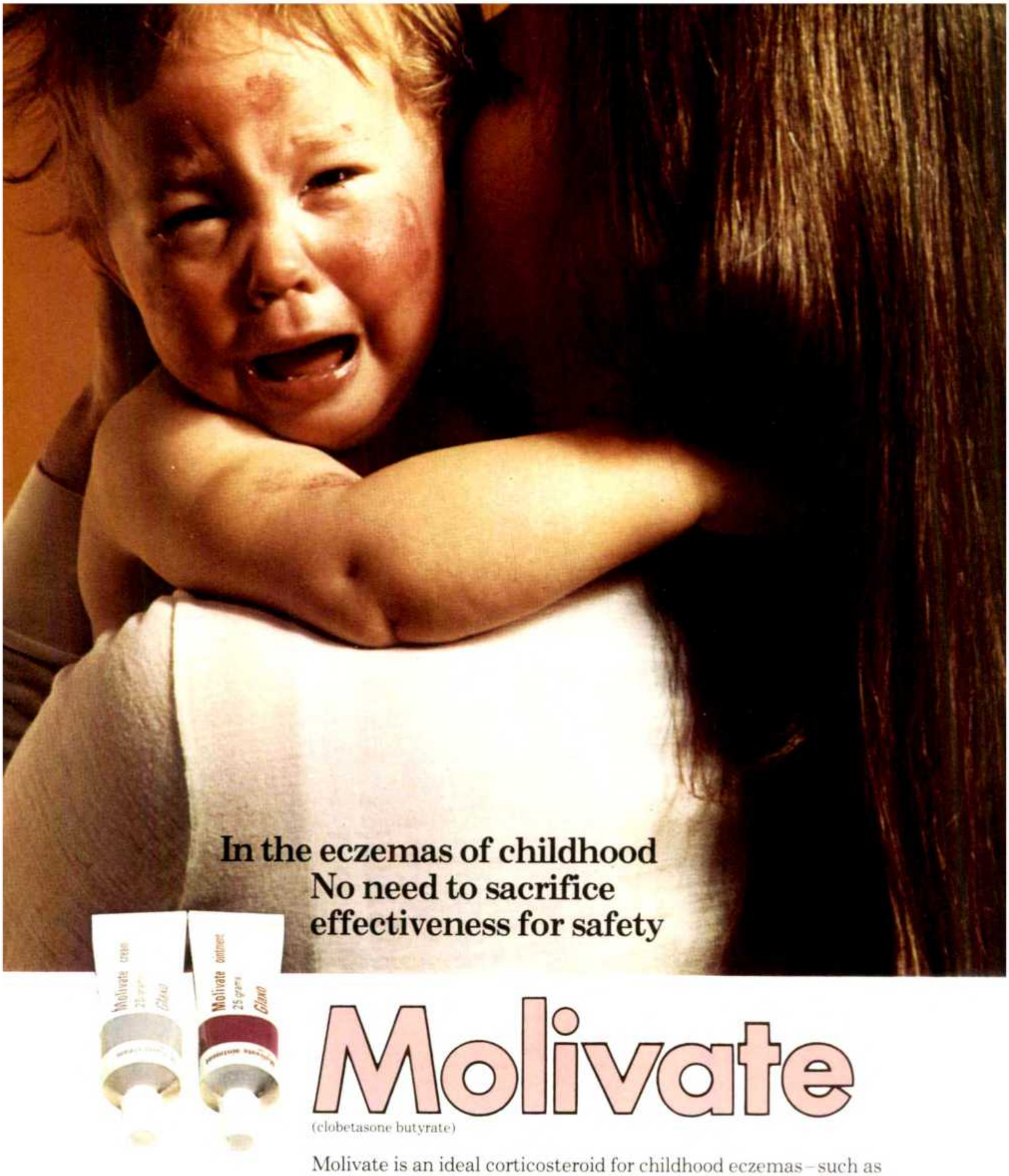
Dermatologists will appreciate DITHROLAN because of the considerable saving in treatment effort and time, and because of the excellent results obtained.

Presentation: DITHROLAN – containing 0·5% Dithranol B.P, 0·5% Salicylic Acid B.P. 90 gram amber glass jar. PL 0173/0024

References: 1. Brit. J. Derm. **93**, *205, 1975.*
2. Dermatology Digest **Feb**, *20, 1976.*

Further information and
literature available from:
Dermal Laboratories Limited,
Tatmore Place, Gosmore, Hitchin,
Herts, SG4 7QR, England

Dithrolan



Molivate

(clobetasone butyrate)

Molivate is an ideal corticosteroid for childhood eczemas – such as atopic eczema – because it has been shown to be significantly more effective than hydrocortisone, yet to have a *wide margin of safety*.*

Cream – particularly suitable for the wet and weeping lesion.
Ointment – for the dry lichenified lesion.

British Medical Journal* (1975) **3, 626

Molivate is available in 25 and 100 gram tubes

Glaxo

Full prescribing information on Molivate (trade mark) is available from Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE

ano-genital pruritus, intertrigo, nappy rash – special skin problems that need the special properties of Timodine.

The special problems of skin-fold dermatoses are those caused by warmth and moisture: maceration and chafing, leading to secondary bacterial and fungal infection, with severe inflammation and pruritus.

Timodine offers a comprehensive formula to counter these problems: nystatin and benzalkonium chloride for antibacterial and anti-fungal effect, hydrocortisone to reduce inflammation and pruritus without the hazards of fluorinated steroids, and dimethicone 350 to protect the skin from moisture.

Timodine
every time

Timodine contains (w/w) Dimethicone 350 B.P.C. 10%, Nystatin B.P. 100,000i.u./g, Benzalkonium Chloride Solution B.P. 0.2%, Hydrocortisone B.P. 0.5%. Tubes of 30g. PL 1839/0001.

Full prescribing information is available.
Lloyd-Hamol Ltd. A member of Reckitt & Colman
Pharmaceutical Division, Hull, HU8 7DS.
Timodine is a registered trade mark.

POTABA helps to straighten out the scleroderma patient. A marked easing of contractive features occurs when scleroderma patients take Potaba. Tests have shown that, when the use of corticosteriods and various other treatments have failed, Potaba brings considerable cutaneous improvement.

Potaba* has been well tried over many years and is a proven aid in the easing of scleroderma, dermatomyositis and many other fibrotic diseases.

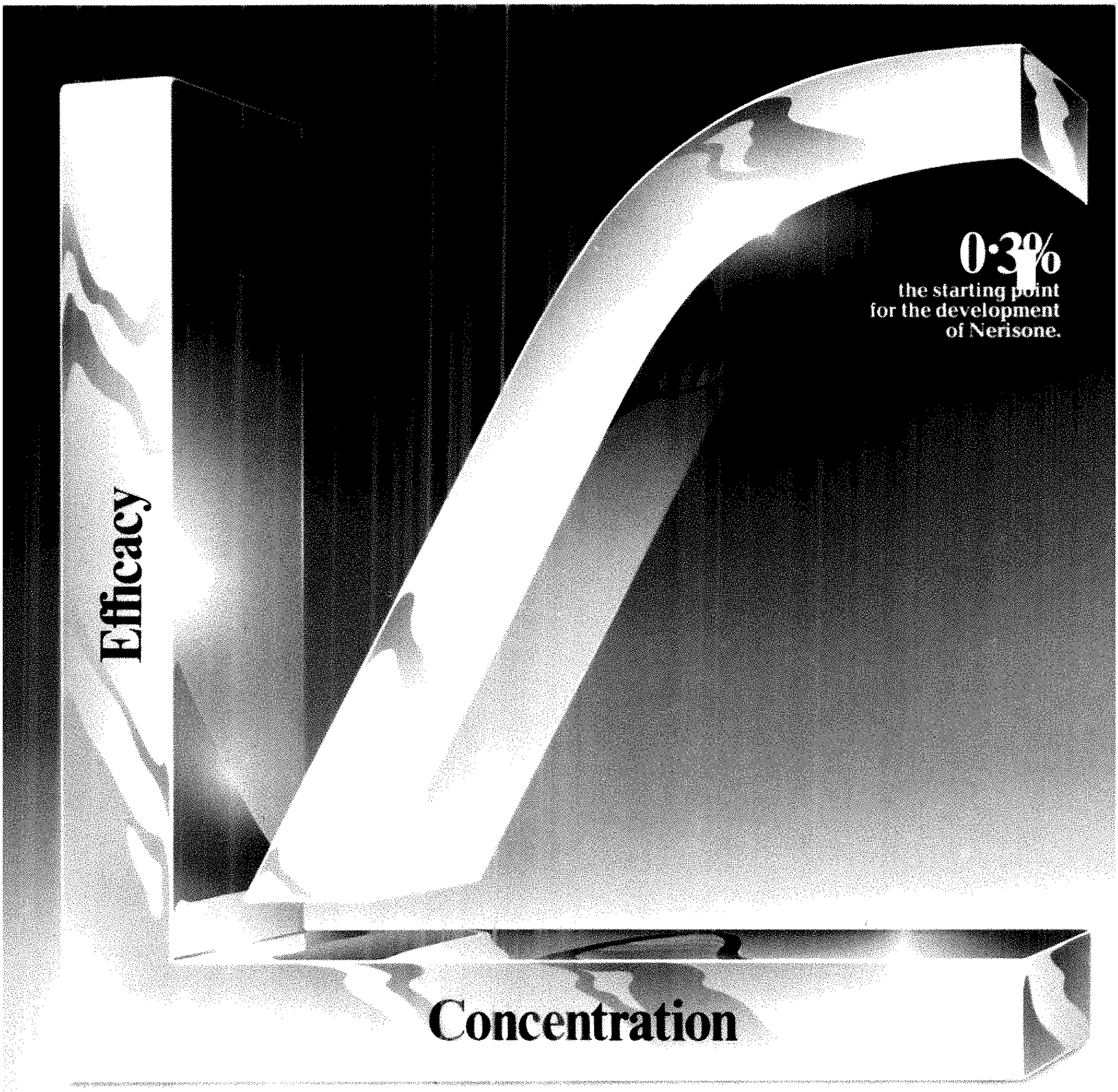
Potaba is presented in:
Envules – Box of 40 sachets (3G)
Tablets – 120 and 1000 (500mg)
Capsules – 240 and 1000 (500mg)
Send for further details and data sheet to
GLENWOOD LABORATORIES LTD., 19 Wincheap,
Canterbury, Kent.
Telephone (0227)
60139

GLENWOOD

*Potassium Para-aminobenzoate (KPAB)

POTABA + 6 includes Pyridoxine Hydrochloride

SCHERIN
A NEW APPROAC



Graph showing the relationship between efficacy and corticoid concentration with diflucortolone valerate.

Product Information
Presentations Cream (oil-in-water emulsion), oily cream (water-in-oil emulsion) and ointment (anhydrous base).
1 gram of each Nerisone presentation contains 1mg (0.1%) diflucortolone valerate. *
Uses Corticoid-responsive dermatoses in the absence of infection.
Dosage and administration Initially 2–3 applications daily according to the severity of the condition. For maintenance one application daily.

Nerisone is available in three bases. The base chosen for an individual case should depend on the physical characteristics of the lesion. For example, in very dry and chronic skin conditions, the occlusive effect of the Nerisone ointment base may often aid the healing process.
Contra-indications, warnings, etc. *Contra-indications* Cutaneous syphilis and tuberculosis in the treatment area, smallpox, chicken-pox and vaccinia. Nerisone is not suitable for the treatment of ophthalmic conditions.

Warnings/Side effects Topical administration of corticosteroids to pregnant animals can cause abnormalities of foetal development. The relevance of this finding to human beings h not been established; however, topical steroids should not be used extensively during the first trimester of pregnancy, i.e. in large amounts or for prolonged periods.
As with all topical corticoids, both local and systemic side-effe can occur in certain circumstances.
Prolonged exposure (e.g. for more than 4 weeks) may lead to

BRING TO SKIN THERAPY

Neither too strong nor too weak, Nerisone has been specifically developed for everyday cases of eczema and dermatitis since these do not need superfluously strong products nor will they respond quickly to the limited efficacy of hydrocortisone products.

Nerisone-developed from the most effective concentration

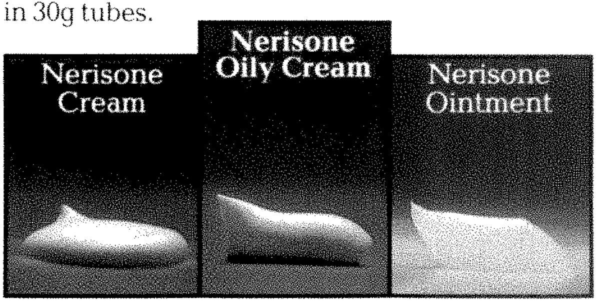
In a widely used dermatological test[1] diflucortolone valerate at a concentration of 0·3% produced more positive results than probably the most potent topical corticoid to date; the real development was to reduce the concentration progressively to 0·1% to arrive at Nerisone which thus has a desired balance between efficacy and safety.

Nerisone-controlled efficacy balanced with excellent tolerance

World-wide clinical trials[2] involving 6,500 patients have shown Nerisone to be over 90% effective in the treatment of eczema and dermatitis while maintaining excellent local and systemic tolerance.

Nerisone-a new base, one tube size

In addition to the conventional cream and ointment bases, new Nerisone is also available in a special OILY CREAM base which combines the softening and occlusive qualities of an ointment with the cosmetic acceptability of a cream. To simplify prescribing, Nerisone is only available in 30g tubes.



Neither too strong nor too weak

NEW Nerisone

balanced to avoid the extremes of corticoid therapy

Schering Chemicals Ltd., Pharmaceutical Division,
Burgess Hill, West Sussex RH15 9NE.

local effects such as atrophy of the skin, striae, telangiectasia or acneform changes, espically when the ointment base is used, or Nerisone is applied under occlusion.
When large areas are treated (again especially with the ointment base or under occlusion), natural steroid production may be depressed. The following precautions should therefor be observed:

1) Areas larger than 10% of the body surface should not be treated for more than four weeks.
2) Occlusive dressings should not be left on for more than 24 hours. They may be used repeatedly, but it should be noted that the risk of systemic effects is thereby increased.
3) If secondary infection occurs during treatment, the use of occlusive dressings should be stopped until the infection has been eliminated.
4) Children up to the age of 4 years should not be treated with Nerisone for longer than three weeks–particularly on skin areas covered by napkins.

Pharmaceutical precautions Store in cool, dry conditions.
Legal category TSA
Package qualities 30g tubes
N.H.S. Price 30g: £1.49
Further information Available on request
Product licence numbers Nerisone Cream 0053/0075 Nerisone Oily Cream 0053/0073 Nerisone Ointment 0053/0074

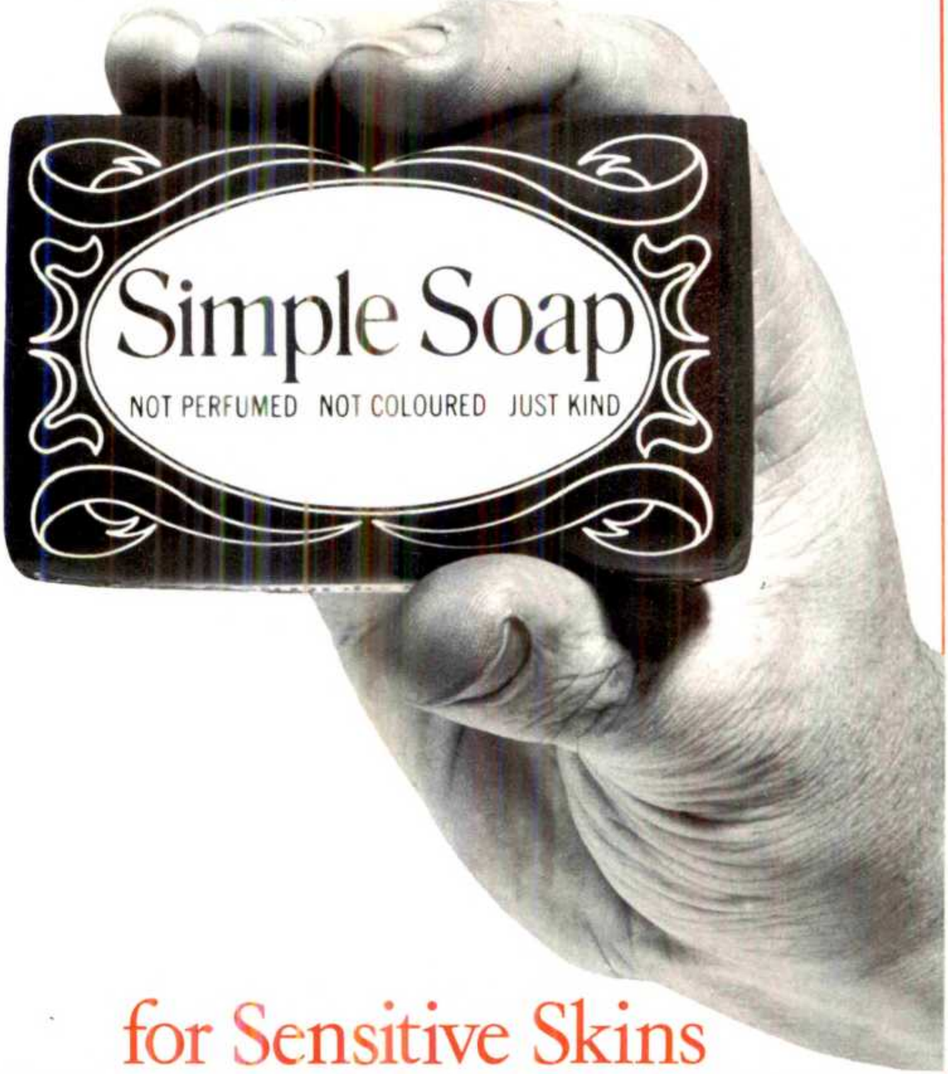
---

[1] *Lofferer O., Rev. Venez. Derm., 14, 1, 1975.*

[2] *Further information available from Schering Chemicals Limited.*

When there is potential danger of skin conditions recurring or being exacerbated through irritation or allergic reaction . . . . . .

Simply recommend



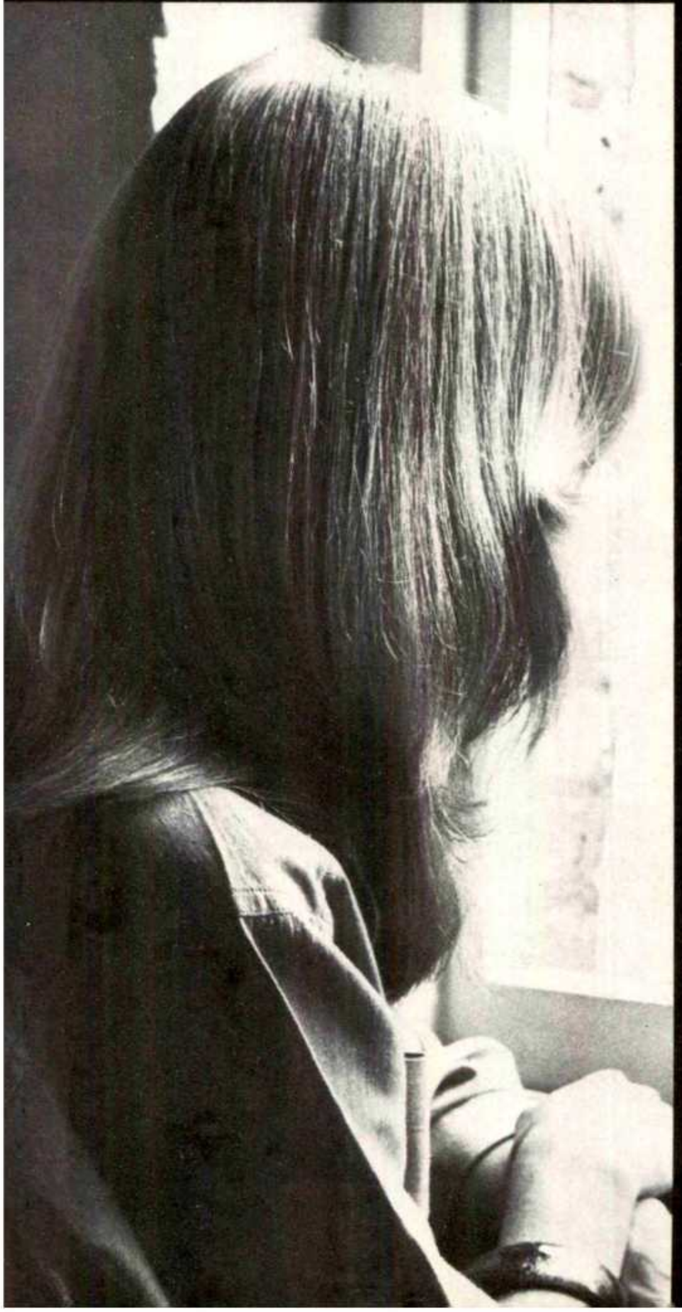
for Sensitive Skins

Skin problems may often be exacerbated by the colourants, perfumes or high-lather detergents contained in many brands of 'cosmetic' soap. Simple Soap contains none of these potentially harmful additives—it simply cleanses with the gentle lathering action of pure soap. That's why Simple Soap is widely used by hospitals throughout the country.

Simple Soap is available to your patients at chemists everywhere, for about 14p and 24p.

A regular supply of professional samples may be obtained on application to:
The Medical Division, Simple Soap Ltd., Hampton, Middlesex.

She's been to the chemist she's been to her doctor now she's come to you



In acne "... not all patients respond satisfactorily [to tetracycline] and there is a need for more effective or alternative therapy."

SEPTRIN* "... has been clinically effective in patients who did not respond to long-term tetracycline."

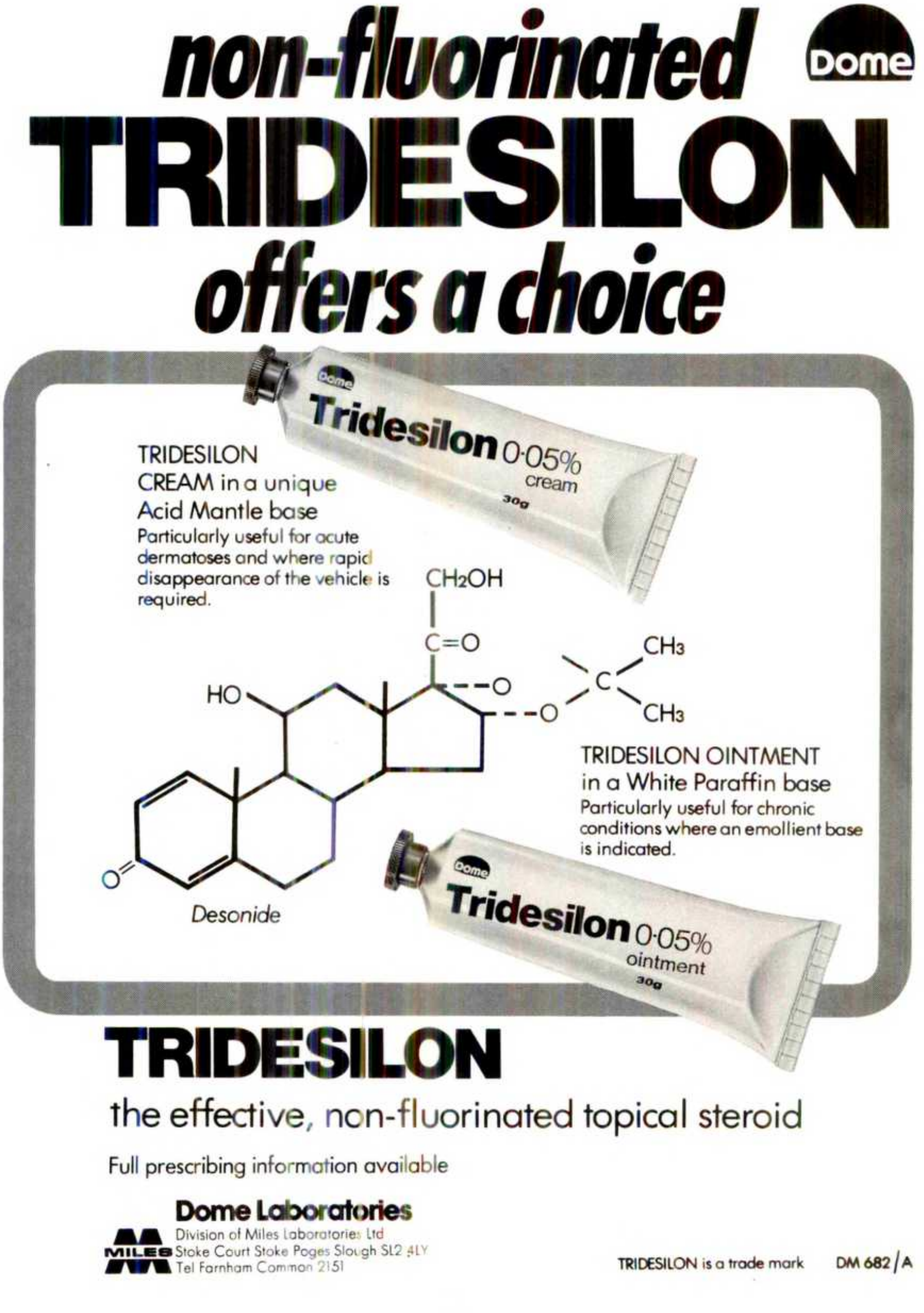
Brit J Derm. (1971), **84**, 366.

SEPTRIN FOR ACNE

Wellcome

Septrin contains trimethoprim and sulphamethoxazole. Full prescribing information is available on request.
Wellcome Medical Division
The Wellcome Foundation Ltd.
Berkhamsted, Herts

*Trade Mark



non-fluorinated TRIDESILON offers a choice

TRIDESILON
CREAM in a unique
Acid Mantle base

Particularly useful for acute dermatoses and where rapid disappearance of the vehicle is required.

Desonide

TRIDESILON OINTMENT
in a White Paraffin base

Particularly useful for chronic conditions where an emollient base is indicated.

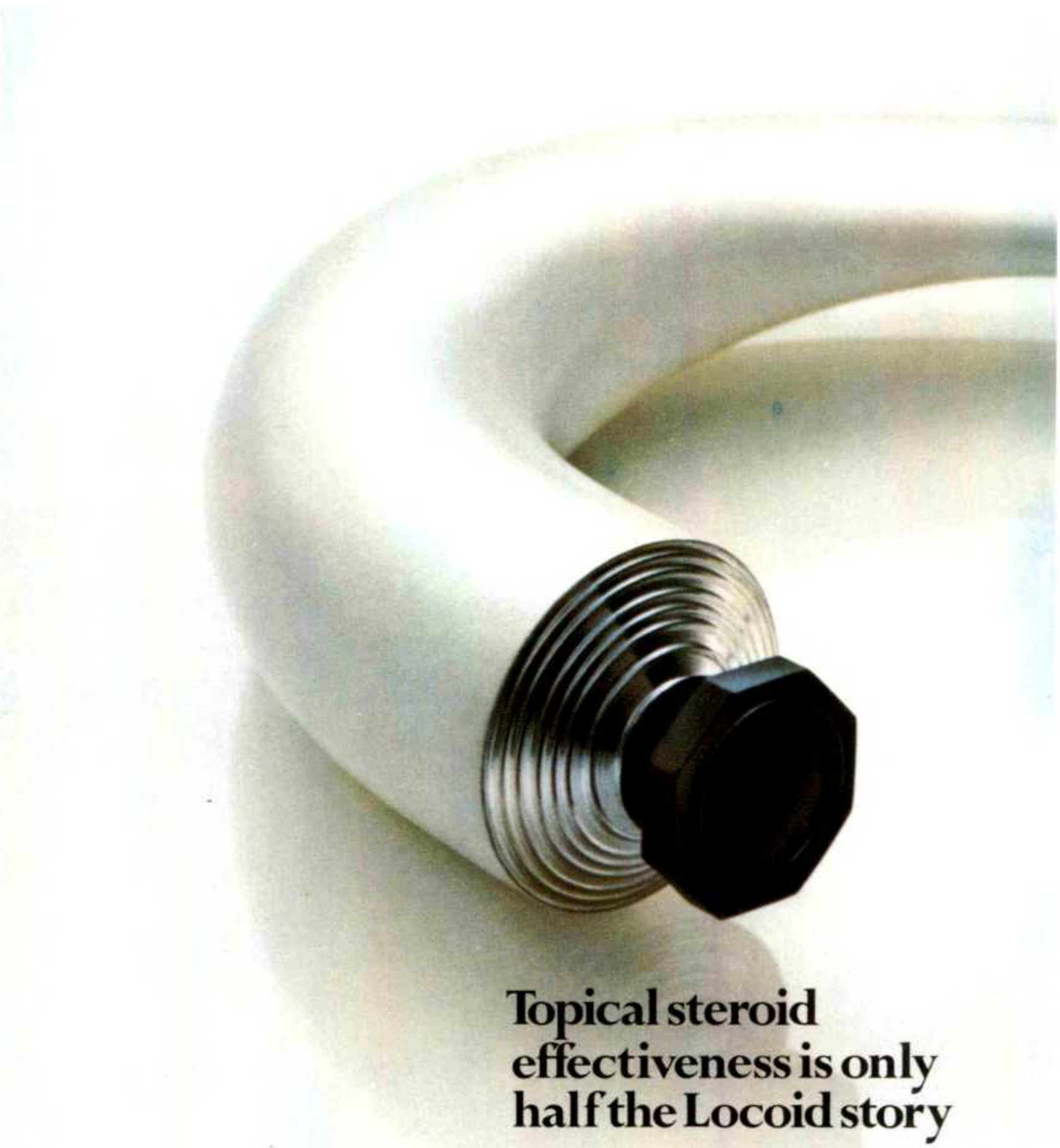
TRIDESILON

the effective, non-fluorinated topical steroid

Full prescribing information available

Dome Laboratories
Division of Miles Laboratories Ltd
Stoke Court Stoke Poges Slough SL2 4LY
Tel Farnham Common 2151

TRIDESILON is a trade mark DM 682/A



Topical steroid effectiveness is only half the Locoid story

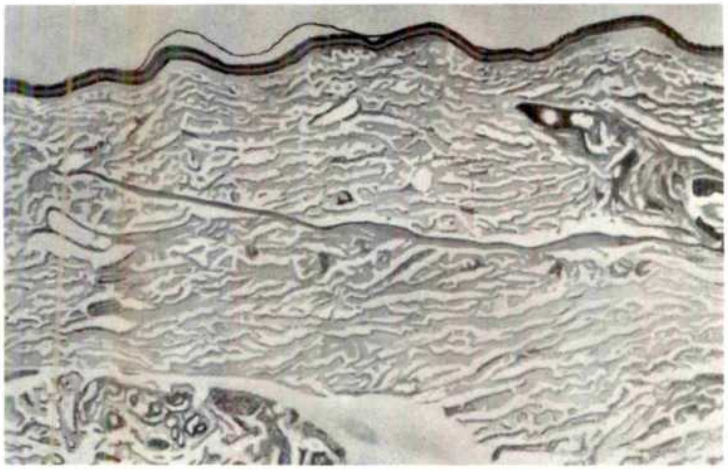
...the other half is the relative freedom from topical side effects which are often encountered with the fluorinated steroids. Locoid offers the same level of success as these potent preparations – without fluorination.

Locoid is a trade mark for hydrocortisone 17-butyrate.
Full information available on request:
Brocades (Great Britain) Ltd.,
Brocades House, West Byfleet, Surrey, KT14 6RA.
Telephone: Byfleet 45536.

locoid

the non-fluorinated way to topical steroid success

In pruritic anogenital conditions to lessen the risks of topical corticosteroid treatment...



...oral 'Vallergan' concurrently

skin atrophy induced by topical corticosteroid application: general histological appearance

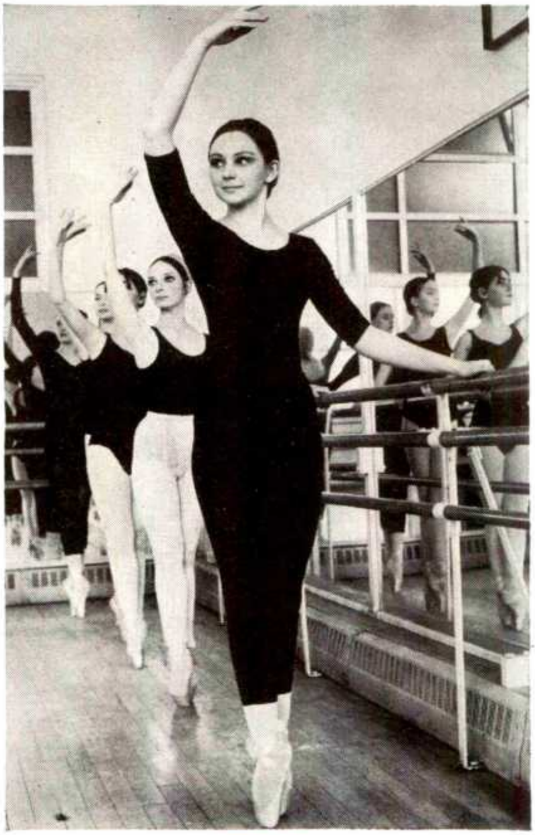
Corticosteroid creams and ointments are accepted and effective treatment for most local inflammatory processes and disorders of the anogenital region, such as dermatoses, haemorrhoids, pruritus and vulvitis, many of which are characterized by chronic itching.
Prolonged and intensive use of these preparations may however produce marked atrophic changes in the skin, with thinning and increased fragility, purpura and striae. There is also the possibility of percutaneous absorption and resultant adrenal suppression.
Adding 'Vallergan' to the regime can help minimize these risks.
'Vallergan' is a potent oral antipruritic agent which rapidly controls itching and scratching. As such, when co-prescribed it can reduce both the frequency and the overall duration of topical corticosteroid therapy. Once applied, the cream or ointment is not rubbed away by the patient but allowed time to exert its full anti-inflammatory effect. As a result fewer applications are required, exacerbation of the condition is avoided, and resolution achieved sooner. All of which means shorter, less intensive treatment, giving steroid benefits with less chance of adverse reactions.
'Vallergan' also has a mild sedative action, helpful in relieving the emotional disturbance and sleeplessness which often accompany pruritic conditions.

'Vallergan'* is supplied as tablets, syrup and Forte syrup. Full prescribing information on request. *trade mark of May & Baker Ltd Dagenham Essex RM10 7XS for its preparations of trimeprazine tartrate

M&B May&Baker A member of the Rhône-Poulenc Group of Companies

MA 4531

ACNE?



prescribe patient confidence

"Acne is an almost universal complaint in adolescence."[1]

"Many patients when they seek the help of their doctor are depressed, discouraged and disillusioned. Their own efforts at treatment have failed, or the first prescription was not the cure they anticipated. In the management of acne the patient must appreciate that he or she may have to persevere with treatment throughout their teens, and it should be emphasised that in this way one hopes to limit as far as possible the severity of the lesions and prevent severe or permanent scarring."[2]

When patients like these ask for your help remember that clinical reports show that acne responds to Quinoderm.

Available as Quinoderm
with **Hydrocortisone Cream 30G.** for initial treatment.
Quinoderm Cream 25G. for subsequent treatment.

1, 2, Practitioner 1970, 204, 639

Quinoderm Hydrocortisone 30G.
Benzoyl Peroxide 10.0%
Potassium Hydroxyquinoline, Sulphate 0.5%
Hydrocortisone 1%
in an astringent cream base.

Quinoderm Cream 25G.
Benzoyl Peroxide 10.0%
Potassium Hydroxyquinoline Sulphate 0.5%
in an astringent cream base.

Quinoderm

For further information and literature please write to THE QUINODERM COMPANY, OLDHAM, LANCS.

tarcortin

Cream

succeeds in eczema where others fail

Tarcortin Cream, a non-staining combination of a highly refined coal tar extract and hydrocortisone, produces excellent results in eczema and dermatitis – even where there has been no response to either agent alone. It has been found highly effective in the treatment of delicate skins.

Tarcortin Cream is available in 30g and 45g tubes.

Further information, including Data Sheet, available on request from:

Stafford-Miller

The Professional Relations
Division, Stafford-Miller
Limited, Hatfield, Herts.

British Journal of Dermatology (1976) **95**, 459.

Clinical and Laboratory Investigations

The sunburn cell in mouse skin: preliminary quantitative studies on its production

A. WOODCOCK AND I. A. MAGNUS

Department of Photobiology, Institute of Dermatology,
Homerton Grove, London E9 6BX

Accepted for publication 2 April 1976

SUMMARY

Quantitative studies on sunburn cell (SBC) production in mouse epidermis are reported. Ultraviolet radiation (UVR), 260–300 nm, produced a dose related effect with respect to SBC formation. Maximal spectral reactivity was at wavelengths shorter than 300 nm. In 8-methoxypsoralen (8-MOP) photosensitization, SBCs were also produced and this response is dose related with respect to long wave UVR dose. In fluorescein photosensitization, provoked by 487 nm light, SBCs were not produced. The response in 8-MOP photosensitization and 254 nm UVR (given alone) differed in respect of time course, but it is considered that both may perhaps be initiated by a DNA lesion.

The 'sunburn cell' (SBC) appears in the human epidermis after exposure to ultraviolet radiation (UVR). It is regarded as a form of individual cell shrinkage necrosis or dyskeratosis and its characteristic histological appearance is well known (Fig. 1). Stained with haematoxylin and eosin, the cell has a pyknotic nucleus and shrunken, glassy, eosinophilic cytoplasm with inter- and intracellular 'oedema' (Hamperl, Henschke & Schulze, 1939; Miescher, 1957; Lorincz, 1960; Daniels, Brophy & Lobitz, 1961). The only quantitative data are the dose–response studies of Johnson, Mandell & Daniels (1972) in humans.

Basic facts required in the characterization of an abnormal response include its time course, dose–response relationship and, in the case of a photobiological reaction, the action spectrum. These are difficult to study satisfactorily in the human SBC and so in this paper we present the results of preliminary work on the mouse. These are mainly for SBC production over the spectral range 260–320 nm UVR. We also report some studies of the role of photosensitizers—8-methoxypsoralen (8-MOP) and fluorescein—in SBC formation.

MATERIALS AND METHODS

Experimental animals, depilation and irradiation

These were male albino haired mice of an established in-bred strain of the Institute of Dermatology, 5–7 weeks old, weighing 26–37 g at the time of the experiments. Before irradiation (usually $\frac{1}{2}$ h) the flank coat was clipped and a commercial depilatory cream ('Nair') applied and washed off after about

LIBRARY

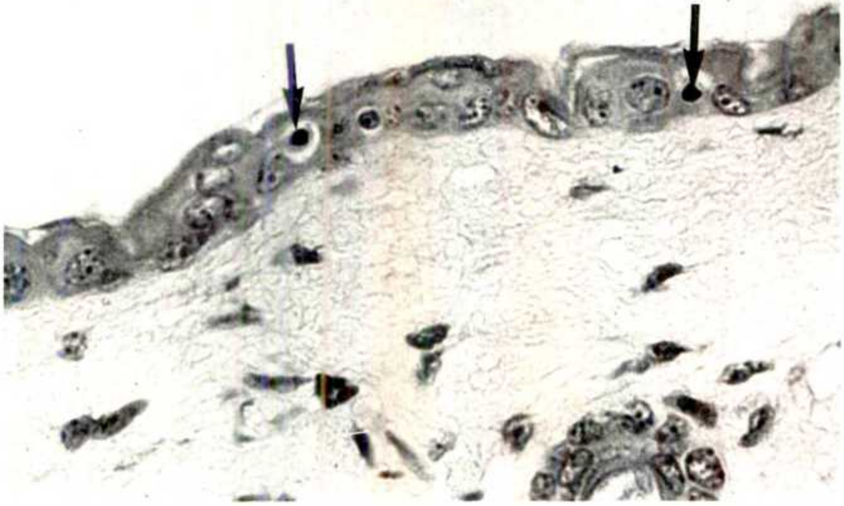


FIGURE 1. Histological appearance of sunburn cell (SBC) in mouse skin (arrows) 8½ h after UVR irradiation (300 nm source), dose 80 mJ/cm² (H & E, × 800).

5 s. Various irradiation sources were then used. The mice were held against an aperture, the 'exit aperture', in a metal plate associated with the optical system and source used. Crystal violet (1% aqueous) from a felt pad surrounding the exit aperture was used to mark the borders of the irradiated skin. The sources were as follows:

Broad waveband short wavelength UVR, principally 290–320 nm, subsequently called the '300 nm source'. Two plano-convex quartz lenses (f = 30 and 50 cm) approximately collimated the radiation from a compact source, 2 kW xenon arc (XE/D, AEI) onto a dichroic mirror (OCLI) at 45° incidence. The reflected radiation, principally shorter than 400 nm, then passed through a narrow band interference filter, peaking at 300 nm (Barr & Stroud). The spectrum at the exit aperture, found with a Beckman DB-G recording spectrophotometer (corrected with a standard source), was of approximately symmetrical bell-shaped profile; it had maximum emission at 300 nm, ½ peak band width *c.* 15 nm, $\frac{1}{10}$ peak band width *c.* 40 nm. An area of skin, approx. 1·0 cm², was exposed at the exit aperture. Irradiance of the skin was *c.* 1 mW/cm².

Broad waveband long wavelength UVR, principally 340–400 nm, subsequently called the 'near UVR source'. Radiation was collimated as above from the xenon arc, UVR below 400 nm reflected from a similar dichroic mirror and directed through a glass lens (f = 11 cm), Wood's glass (3 mm thickness) and a WG 360 Schott glass filter (3 mm thickness). The spectrum at the exit aperture, checked spectrophotometrically as above, was from about 325 to 425 nm, peaking at 375 nm. The area of skin irradiated was *c.* 0·4 cm². Irradiance of the skin was *c.* 2 mW/cm².

An irradiation monochromator with water prism and 2 kW xenon arc source (Magnus *et al.*, 1959). Half band width at 260 nm was about 1 nm, at 320 nm, 2 nm, and at 487 nm (for fluorescein photosensitization), 10 nm. To increase flux homogeneity over the exit slit, a 'spectrosil' field lens (f = 3·0 cm) was positioned immediately beyond this slit. The irradiated area of skin was approx. 0·1 cm². Irradiance of the skin varied from about 0·5 mW/cm^2 at 260 nm, 10 mW/cm^2 at 320 nm and 0·5 W/cm^2 at 487 nm.

A low pressure mercury lamp tube (Philips TUV/30), principally 254 nm UVR, subsequently called the '254 nm source'. The lamp was connected to a mains stabilizer. Irradiation was at an aperture 8 cm away from the mid point of the length of the tube. The lamp was used unfiltered and of the wavelengths shorter than 320 nm, approximately 99% were of 254 nm. The area of skin irradiated was approximately 1·0 cm^2. Irradiance of the skin was approximately 1 mW/cm^2.

Dosimetry. For every experiment, except in that on fluorescein photosensitivity, the irradiance and dose at the skin surface were calculated by potassium ferrioxalate actinometry (Hatchard & Parker, 1956). Dosimetry in the wavelength region used in fluorescein photosensitization (around 487 nm) was unsatisfactory with the actinometer and a calibrated Hilger and Watts quartz thermopile (FT17) connected to a Farnell digital voltmeter was substituted to estimate irradiance and dose.

Preparation for microscopy and counting of SBCs. Animals were killed by cervical dislocation and skin specimens were excised and placed within 5 min in Bouin's fluid for fixation. After routine processing, 5 μm paraffin sections were cut and stained with haematoxylin and eosin. In each skin specimen SBCs were counted in microscope fields along the length of 10 randomly taken sections. The microscope (× 40 objective and × 10 eyepiece) contained an eyepiece graticule to ensure that the same length of epidermis was enclosed in each field. The number was expressed as SBCs (±s.d.) per cm length of epidermis in each skin specimen. The counts were expressed in this way rather than as a ratio of the total number of Malpighian cells, because UVR induces hyperplasia and thus variation in epidermal cell numbers, also because radiation damage sometimes made identification and counting of individual Malpighian cells difficult. SBCs within hair follicles were ignored.

Photosensitizers. Crystalline 8-MOP (Sigma) was applied topically, dissolved in ethyl alcohol, the coat having been removed ½ h earlier. The fluorescein used (Hopkins & Williams) when dissolved in distilled water had an absorption spectrum peak at about 487 nm. This dye was given by intraperitoneal injection, as topical and oral photosensitization proved unsatisfactory.

EXPERIMENTS

To check that the depilation procedure *without* irradiation did not provoke SBCs, samples of skin were obtained from the flanks of forty-five mice, five mice at each of the following times after depilation: 3, 5½, 9, 18½, 24½, 29½, 32½, 47½ and 71½ h.

SBC production by UVR alone

Time course. 1. 254 nm source, dose 70 mJ/cm^2. Skin specimens were from 35 mice irradiated on both flanks, five mice each at 2½, 8½, 11½, 18, 29, 47 and 71 h after irradiation.

2. 300 nm source, dose 80 mJ/cm^2; a similar experiment was done using 55 mice, five mice each with specimens taken at 2½, 5, 8½, 11½, 18, 24, 29, 32, 47, 58 and 71 h after irradiation.

Action spectra and dose-response relationships

1. One hundred and thirty mice were irradiated on both flanks with an irradiation monochromator, five or more mice at each dose of the following seven wavelengths; 320 nm: 35·5, 355, 710 mJ/cm^2;

310 nm: 15, 50, 150 mJ/cm^2; 300 nm: 5, 15, 50, 150 and 450 mJ/cm^2; 290, 280 and 270 nm: each with 15, 50, 150 mJ/cm^2; 260 nm: 5, 15, 50 mJ/cm^2. The dose ranges were limited by what were practical exposure times with the irradiation monochromator. Skin specimens for histology were taken 8½ h after exposure; five control mice were depilated but not irradiated and skin samples taken 9 h after depilation.

2. An additional study of the action spectrum for SBC production 24 h after irradiation was done on forty-five mice at one dose only, 50 mJ/cm^2. The same seven wavelengths as above were used with at least 5 mice irradiated on both flanks at each wavelength. Again specimens from five unirradiated mice were studied as controls and taken 24½ h after depilation.

8-*MOP: The time course of SBC production.* All manoeuvres, except irradiation, were made under red photographic safety light and the animals kept in darkness. A total of sixty-five mice were used. Thirty mice, after depilation, had two skin areas of 3·75 cm^2 covered with 0·1 ml of a solution of 2 mg/ml 8-MOP in ethanol. The 8-MOP solvent was applied with a graduated pipette to two areas on the flank with the mouse immobilized, until the solution had evaporated. Immobilization was in a vinyl sheet jacket with two windows (3·75 cm^2) cut in it over the treatment areas. One half to ¾ h later, each mouse was irradiated on the treated areas of both flanks with the near UVR source, dose 3 J/cm^2. Ten irradiated areas from five mice were excised for histology at each of the following time intervals: 2½, 8½, 11½, 18½, 24 and 48 h. Specimens of skin, 8-MOP treated but unirradiated, were also taken from each flank as controls. Another thirty mice were irradiated as above but not treated with 8-MOP and specimens were again obtained at the above times. A further five mice were treated topically with ethanol without 8-MOP, irradiated and killed 24 h later for skin histology.

8-*MOP: The dose response for SBCs.* This was studied in skin pretreated with a constant dose of 8-MOP (as above) ½ h before irradiation. Three doses from the near UVR source (0·11, 0·33 and 0·99 J/cm^2) were given to each of nine mice, two exposures on one flank, one on the other flank with a fourth area unirradiated. Specimens for histology were obtained 18 h after irradiation.

Fluorescein and SBCs. Mice were photosensitized with intraperitoneal fluorescein 0·2 ml of 0·05 mg/ml in distilled water. Five normal and three groups each of five to six photosensitized mice were depilated on both flanks and irradiated with a monochromator 30–45 minutes later at 487 nm, dose 30 J/cm^2. Again, except during irradiation, all mice were handled under red light or kept in the dark. Skin specimens were taken at 1, 8 or 24 h after irradiation. As well as skin samples that had been both fluorescein-treated and irradiated, three controls were taken (1) fluorescein treated but unirradiated, (2) irradiated but not fluorescein treated, (3) depilated only.

RESULTS

First the effect of the depilation procedure on SBC production was studied. It was found that removing the coat without irradiation provoked extremely few SBCs.

SBC formation by UVR only

Fig. 2, indicates the time course of SBC production with 70 mJ/cm^2 from the 254 nm source. By 2½ h after irradiation, SBCs appeared and the maximum number of cells was attained by about 8 h. Thereafter, the number declined and from 24 to 48 h, the epidermis was generally too damaged for satisfactory counting. Dermal SBCs from 254 nm irradiation were not observed.

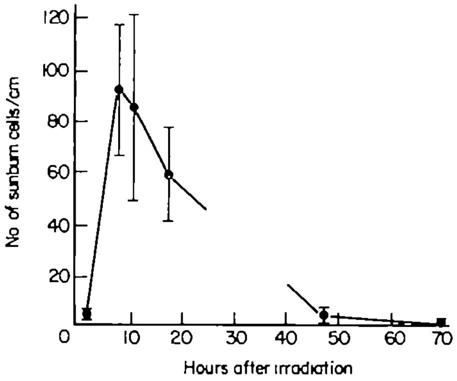


FIG. 2.

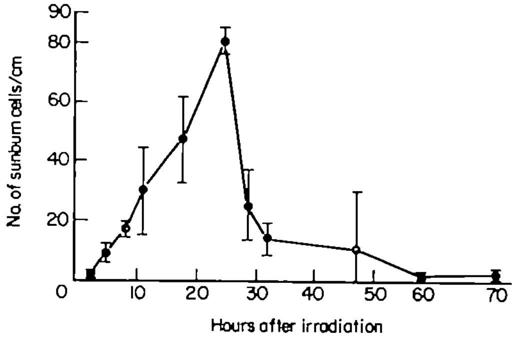


FIG. 3.

FIGURE 2. Time course for SBC production after 70 mJ/cm^2 from 254 nm source. Mean values ± s.d. At 24–40 h after irradiation, epidermal damage precluded satisfactory SBC counting.

FIGURE 3. Time course for SBC production after 80 mJ/cm^2 from 300 nm source. Mean values ± s.d.

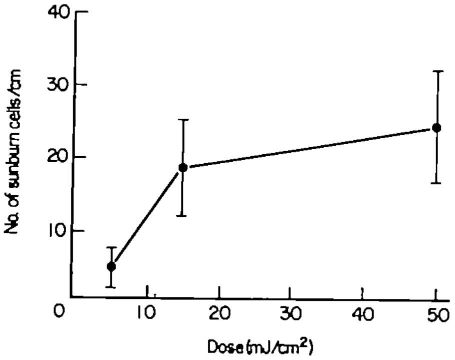


FIGURE 4. Dose response curve for SBC production after monochromatic 260 nm UVR. Mean values ± s.d.

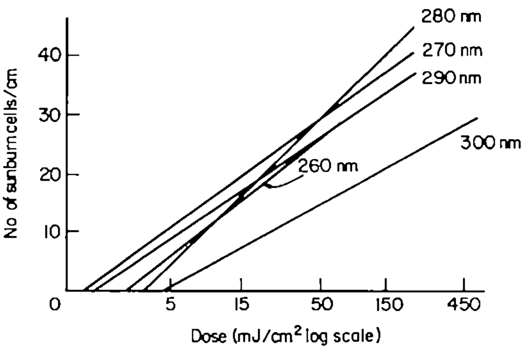


FIGURE 5. Composite of regression lines for dose-response curves for SBC production by monochromatic UVR 260 to 300 nm. See text.

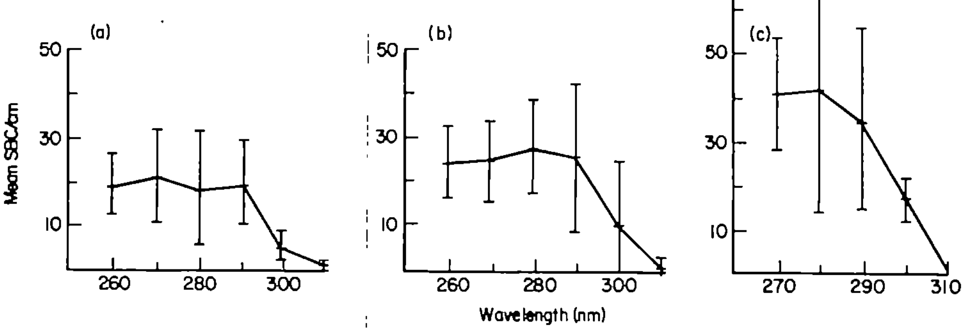


FIGURE 6. Action spectra for SBC production by monochromatic UVR. Mean values ± s.d. Counts made 8½ h after irradiation. (a) dose = 15 mJ/cm^2; (b) dose = 50 mJ/cm^2; (c) dose = 150 mJ/cm^2.

Fig. 3 indicates that, after a 80 mJ/cm^2 dose from the 300 nm source, SBCs were detected by about 5 h after irradiation, but the number of SBCs subsequently increased slowly to reach a maximum about 24 h after irradiation; the number thereafter declined. By 60 h after irradiation, the epidermis was overcome by widespread damage and SBCs were difficult to identify, although seemingly low in number. A few SBCs were observed in the dermis in this experiment.

Fig. 4 is the dose–response curve obtained for SBCs by irradiation with 260 nm UVR; similar curves were obtained with 270–300 nm, but with 310 and 320 nm the response was insignificant.

Plotted with respect to log dose, the curves seem to have a straight line relationship. Fig. 5 shows regression lines for these log dose–response curves. Thus with irradiation with 260–300 nm, SBC

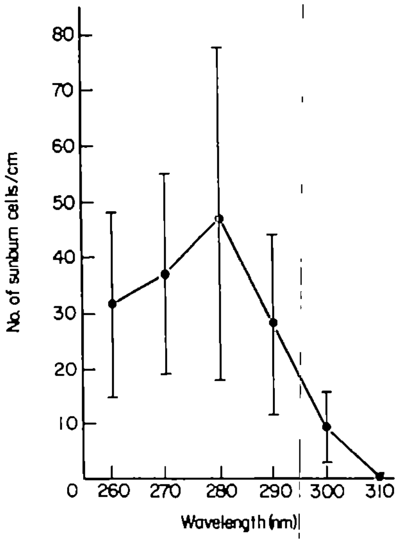


FIG. 7.

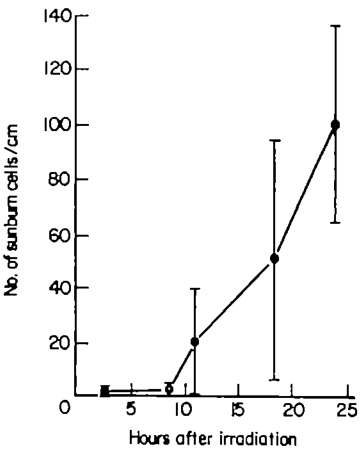


FIG. 8.

FIGURE 7. Action spectra for SBC production by monochromatic UVR. Mean values ± s.d. Counts made 24 h after irradiation. Dose = 50 mJ/cm^2.

FIGURE 8. Time course for SBC production in skin treated topically with 8-MOP. Irradiation ½ h later with near UVR source. Mean values ± s.d.

production is dose related (positive correlation between dose and response, $P<0{\cdot}05$), and there is no statistically significant difference between the slope of each of these lines.

Fig. 6a–c, shows the same data as action spectra for SBC production, 8½ h after irradiation with doses of 15, 50 and 150 mJ/cm^2. It is seen that the shorter wavelengths are active and that there is no significant difference between the results at the spectral points 260–290 nm. The wavelength 300 nm is however probably less effective and 310 nm has very much less effect. The results for the action spectrum, dose 50 mJ/cm^2, measured at 24 h is shown in Fig. 7. The responses are essentially the same as in Fig. 6.

SBC formation in 8-*MOP photosensitization*

The histological appearance in the light microscope of SBCs provoked by treatment with this psoralen and irradiation with the near UVR source seems to be identical to that of SBCs provoked by shorter wavelength UVR alone.

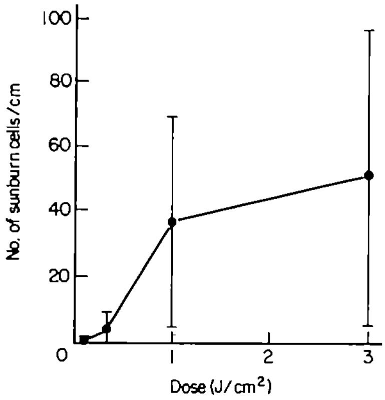


FIGURE 9. Dose-response curve for SBC production after 8-MOP topical treatment. Irradiation ½ h later with near UVR source. Mean values ± s.d.

In Fig. 8, the time course of 8-MOP provokation shows few SBCs up to 8½ h after irradiation. The numbers rose to a maximum at 24 h, but after this epidermal damage precluded SBC counting. The vehicle used for 8-MOP, ethanol, produced a negligible SBC response.

Fig. 9. shows that in 8-MOP photosensitization, SBC production is dose related with respect to radiation dose. The number of SBCs may perhaps follow a straight line relationship with respect to log-dose, and the regression line for these results shows a significantly increased response with increasing radiation dose ($P<0{\cdot}001$).

Fluorescein. Within 30–40 min of intraperitoneal injection with this dye, the skin of the whole animal turned yellow or orange. Skin sites irradiated with blue–green light, a waveband centred at 487 nm, became white and swollen during or immediately after irradiation. By contrast, after 24 h, the injected mice had lost their generalized orange colour and instead, the irradiated areas became orange. No skin reactions to light were observed in mice irradiated but not given fluorescein. Histologically, the epidermis from the fluorescein-treated, irradiated samples showed oedema, more marked in the dermis. There was no increase in SBC production over the controls with irradiation at 1, 8 and 24 h after giving fluorescein.

DISCUSSION

In the present work, some of the more important factors that have a bearing on SBC formation have been examined. Others have not been taken into account, for instance, the role of the mouse hair cycle has been ignored, and this may well account, in part, for the somewhat large scatter obtained in our results. Nevertheless, we can arrive at some indications as to the mechanism of SBC production, if we cannot provide firm numerical data.

UVR alone and SBC production

The results in our dose–response experiments for SBC formation in mouse skin by monochromatic radiation of wavelengths 260, 270, 280, 290 and 300 nm, suggest a log dose–response relationship which follows a straight line. As the analysis of the regression lines for the dose–response curves for 260–300 nm shows no significant difference in slope, this and the somewhat flat action spectrum between 260 and 290 nm (see below), is compatible with the interpretation that the mechanism for provoking SBCs may be the same whatever the wavelength over this spectral range. If the same applies for the human, this might suggest that SBC and erythema production by UVR have different mechanisms (van der Leun, 1972).

In an action spectrum, maximal activity at 260 nm UVR would suggest nucleic acid as the primary radiation absorber, whereas at 280 nm would suggest protein. But we found that activity seemed to be similar at both these wavelengths and unfortunately, irradiation with shorter wavelengths, e.g. 230 and 240 nm, was not feasible with the monochromators available, owing to insufficient radiant flux. The tentative conclusion would be that the results of our action spectrum studies are consistent with UVR acting on either nucleic acid and/or protein with respect to SBC formation.

The time course after 254 nm UV irradiation shows a rise and then a fall in the number of SBCs; this suggests that, after being formed, SBCs are capable of being either restored to normal or removed. We can suppose, therefore, that the *mean* 'life time' of a SBC under these conditions must be shorter than 8 h, as the maximum SBC count is at this time. By contrast, the SBC response time-course to 300 nm UVR might depict a more gradual process of cell damage than that obtained with 254 nm radiation, if we neglect the small differences in dose used and any differences in transmission by the skin of these two wavelengths.

According to Kerr, Wyllie & Currie (1972) the SBC in skin is an example of shrinkage necrosis for which they give the term 'apoptosis'. They look upon this as a process of 'cell deletion' or 'controlled cell death', a view which Olson & Everett (1975) support. Apoptosis, it seems, may occur in most tissues, and may be provoked by several very different types of stimulus other than UVR, such as toxins or hypoxaemia. Apoptosis also may play a role in the cell kinetics of growth in neoplasia (Kerr & Searle, 1972a,b). The process of *removal* of apoptotic cells in the liver has been studied by electron microscopy by Kerr *et al.* (1972). This seems to be by phagocytosis, either by neighbouring parenchymal liver cells, or by histiocytes. Whether a similar removal process of SBCs occurs in epidermis we do not know; one would have expected the disintegrated SBC to be lost in the granular or horny layer during the process of keratinization. Although the normal 'transit time' for a cell to pass from the basal to the horny layer in the mouse seems to be about 4 days (Potten, 1975), this length of time may well be reduced by UV irradiation. Occasionally we observed SBCs in the necrotic epidermis prior to this being sloughed off, but the methods used in the present work do not allow us to come to any conclusion as to the final fate of these cells. There remains the possibility that, in some earlier stage of their development, SBCs could revert to normal, i.e. are 'repaired'. But we have no evidence that they may be subject to any repair process, as, at a molecular level, are some UVR-induced DNA lesions, e.g. pyrimidine dimers (Smith, 1971).

8-MOP photosensitization and SBC production. The photosensitizing action of certain psoralens is well established (see Musajo *et al.*, 1974). The action spectrum for erythema in skin caused by 8-MOP lies within 320–380 nm; this has been confirmed by many different workers, most recently by Nakayama *et al.* (1974). That this spectral range produces SBCs in 8-MOP photosensitization in mouse skin seems first to have been noted by Pound (1970), a finding which we have confirmed. We also found that SBC production by 8-MOP is dose related with respect to radiation dose given within this spectral range.

The time course of SBC production in 8-MOP photosensitization is quite different to that observed with 254 nm UVR alone. In 8-MOP photosensitization, onset of SBC production is first seen at about 8 h and its maximum response is at 24 h at the earliest. By contrast, as noted above, with 254 nm UVR alone, the maximum number of SBCs was rapidly reached, i.e. at about 8 h, and subsequently there was a decline. This suggests a different initial mechanism for SBC formation in each instance. We may note in passing that the *erythema* from 8-MOP photosensitization of human skin is similarly delayed as compared with that from 254 nm UVR used alone. However, we have no evidence to connect the formation of the SBC with the appearance of vasodilatation.

If we suppose that in the provokation of the SBC the initial lesion is in DNA, the difference in its time course may be due to the differing types of DNA lesions in 254 nm alone and in 8-MOP photosensitization. Photosensitizing psoralens form photoadducts with pyrimidine bases of DNA (Rodighiero *et al.*, 1970) and interstrand links between the DNA double helix (Cole, 1971); 254 nm UVR alone forms pyrimidine dimers and other lesions such as protein-DNA interaction (Smith, 1971). Interestingly, cyclophosphamide, a DNA cross-linking agent, seems to produce cells in the epidermis which, as illustrated by Milne (1972), resemble SBCs.

Fluorescein photosensitization and SBC production. The main macroscopic effects in skin photosensitization with fluorescein in the mouse seem to be vascular, i.e. dermal, rather than epidermal; although we were able to provoke photosensitization, as evidenced by an immediate oedematous skin response on irradiation with wavelengths that this dye absorbs (blue–green light) we were not able to detect SBCs. Fluorescein skin photosensitization is almost certainly of photodynamic type, and Allison, Magnus & Young (1966) have supposed that such agents photosensitize by damaging cell membranes or organelles such as lysosomes. Takase, Nijo & Konishi (1974) have reported the microscopic changes of cells in culture, photosensitized by certain xanthene dyes, including two types of fluorescein dye, and found evidence of cytoplasmic rather than nuclear damage. So perhaps with fluorescein the main effect is not on the epidermal cell nucleus and this may be why SBCs seem not to be a feature in photosensitization by this chemical.

The significance of the SBC. This is not known. However, our results suggest the possibility that the initial photochemical event in SBC provokation may be a DNA or protein lesion or both. As already mentioned, Kerr *et al.* (1972) have suggested that the SBC is an example of a 'cell deletion' mechanism, for which they have proposed the term apoptosis. According to them this phenomenon can be induced by a variety of stimuli; the fact that UVR is one of these may be very convenient as a research tool. Using a fluorescent antibody technique, Tan, Freeman & Stoughton (1970) studied the limits of the action spectrum for the production of DNA lesions by irradiation of hairless mouse epidermis. Although their method was not strictly quantitative, their results suggest that wavelengths shorter than 290 nm were the more active, those longer almost without effect. Correlation of SBC production with degree of DNA damage by a technique such as above would be an obvious avenue for further research. Such an approach may, therefore, provide a much needed method for linking lesions at the molecular level with those at the histological and contribute valuable information in this field.

ACKNOWLEDGMENTS

Thanks are due to Miss E. Linton for help in some of the experiments, to the Dunhill and Wellcome Trusts for financial assistance and to Drs A.Jarrett and B.E.Johnson for helpful criticism.

REFERENCES

ALLISON, A.C., MAGNUS, I.A. & YOUNG, M.R. (1966) Role of lysosomes and cell membranes in photosensitization. *Nature (London)*, **200**, 874.

COLE, R.S. (1971) Psoralen monoadducts and inter-strand cross-links in DNA. *Biochimica et Biophysica Acta*, **254**, 30.

DANIELS, F. JR., BROPHY, L. & LOBITZ, W. (1961) Histochemical responses of human skin following ultraviolet irradiation. *Journal of Investigative Dermatology*, **37**, 351.

HAMPERL, H., HENSCHKE, U. & SCHULZE, R. (1939) Vergleich der Hautreaktionen beim Bestrahlungserythem und bei der direkten Pigmentierung. *Virchows Archiv für pathologische Anatomie und Physiologie*, **304**, 19.

HATCHARD, C.G. & PARKER, C.A. (1956) A new sensitive chemical actinometer. II. Potassium ferrioxalate as a standard chemical actinometer. *Proceedings of the Royal Society A*, **235**, 518.

JOHNSON, B.E., MANDELL, G. & DANIELS, F. (1972) Melanin and cellular reactions to ultraviolet radiation. *Nature (New Biology)*, **235**, 147.

KERR, J.F.R. & SEARLE, J. (1972a) A suggested explanation for the paradoxically slow growth rate of basal-cell carcinomas that contain numerous mitotic figures. *Journal of Pathology*, **107**, 41.

KERR, J.F.R. & SEARLE, J. (1972b) The digestion of cellular fragments within phagolysosomes in carcinoma cells. *Journal of Pathology*, **108**, 55.

KERR, J.F.R., WYLLIE, A.H. & CURRIE, A.R. (1972) Apoptosis. A basic biological phenomenon with wide ranging implications in tissue kinetics. *British Journal of Cancer*, **26**, 239.

LORINCZ, A.L. (1960) Physiological and pathological changes in skin from sunburn and suntan. *Journal of the American Medical Association*, **173**, 1227.

MAGNUS, I.A., PORTER, A.D., MCCREE, K.J., MORELAND, J.D. & WRIGHT, W.D. (1959) A monochromator. An apparatus for the investigation of the responses of the skin to ultraviolet, visible and near infra-red radiation. *British Journal of Dermatology*, **71**, 261.

MIESCHER, G. (1957) Zur Histologie der lichtbedingten Reaktionen. *Dermatologica*, **115**, 345.

MILNE, J.A. (1972) *An Introduction to the Diagnostic Histopathology of the skin*, p. 48. Edward Arnold, London.

MUSAJO, L., RODIGHIERO, G., CAPORALE, G., DALL'ACQUA, F., MARCIANI, S., BORDIN, F., BACCICHETTI, F. & BEVILACQUA, R. (1974) Photoreactions between skin-photosensitizing furocoumarins and nucleic acids. In: *Sunlight and Man* (Ed. by T.B.Fitzpatrick, M.A.Pathak, L.C.Harber, M.Seiji and A.Kukita), p. 369. University of Tokyo Press, Japan.

NAKAYAMA, Y., MORIKAWA, F., FUKUDA, M., HAMANO, M., TODA, K. & PATHAK, M.A. (1974) Monochromatic radiation and its application—laboratory studies on the mechanism of erythema and pigmentation induced by psoralen. In: *Sunlight and Man* (Ed. by T.B.Fitzpatrick, M.A.Pathak, L.C.Harber, M.Seiji and A. Kukita), p. 591. University of Tokyo Press, Japan.

OLSON, R.L. & EVERETT, M.A. (1975) Epidermal apoptosis: Cell deletion by phagocytosis. *Journal of Cutaneous Pathology*, **2**, 53.

POTTEN, C.S. (1975) Epidermal transit times. *British Journal of Dermatology*, **93**, 649.

POUND, A.W. (1970) Personal communication.

RODIGHIERO, G., MUSAJO, L., DALL'ACQUA, F., MARCIANI, S., CAPORALE, G. & CIAVATTA, L. (1970) Mechanism of skin photosensitization by furocoumarins. *Biochimica et Biophysica Acta*, **217**, 40.

SMITH, K.C. (1971) The roles of genetic recombination and DNA polymerase in the repair of damaged DNA. *Photophysiology*, **6**, 209.

TAKASE, T., NIJO, S. & KONISHI, H. (1974) Protein-combining properties of xanthene dyes in phototoxic cutaneous reactions. In: *Sunlight and Man* (Ed. by T.B.Fitzpatrick, M.A.Pathak, L.C.Harber, M.Seiji and A.Kukita), p. 445. University of Tokyo Press, Japan.

TAN, E.M., FREEMAN, R.G. & STOUGHTON, R.B. (1970) Action spectrum of ultraviolet light induced damage to nuclear DNA *in vivo*. *Journal of Investigative Dermatology*, **55**, 439.

VAN DER LEUN, J.C. (1972) On the action spectrum of ultraviolet erythema. In: *Research Progress in Organic, Biological and Medicinal Chemistry*, Vol. 3, part II, p. 711. North Holland, Amsterdam.

British Journal of Dermatology (1976) 95, 469.

Thymus-dependent (T) lymphocyte deficiency in progressive systemic sclerosis

P.HUGHES, SHIRLEY HOLT, N.R.ROWELL AND JANIS DODD

The University Department of Medicine, The Northern General Hospital, Sheffield S5 7AU, and The University Departments of Dermatology and Pathology, The General Infirmary at Leeds, Leeds LS1 3EX

Accepted for publication 26 April 1976

SUMMARY

Circulating thymus-dependent (T) lymphocytes were estimated in twenty-seven patients with progressive systemic sclerosis (PSS) and in forty-five normal controls using the property of T lymphocytes to form rosettes with sheep red blood cells. The patients with PSS were found to have a reduction of T lymphocytes which correlated with the extent of visceral involvement by the disease, those with the lowest counts having the most extensive disease. These findings support the suggestion that immunological factors may be involved in the pathogenesis of PSS.

Although the aetiology of progressive systemic sclerosis (PSS) remains uncertain, the presence in the disease of lymphocytes sensitized to tissue antigens (Hughes, Holt & Rowell, 1974) and cytotoxic for cultured target cells (Currie, Saunders & Knowles, 1971) suggests that disordered cell-mediated immunity may be involved in the pathogenesis. The present investigation has examined another aspect of cell-mediated immunity in the disorder; the thymus-dependent (T) lymphocyte population of peripheral blood. We have found that patients with PSS have a deficiency of T lymphocytes, which correlates positively with the extent of visceral involvement, thus providing further support for the view that the disease may have an immunological basis.

MATERIALS AND METHODS

Patients. Twenty-seven patients with PSS, two of whom were male, with a mean age of 50·5 ± 13·5 years, were investigated. Only four patients were receiving treatment with prednisolone (7·5–10·0 mg/day). The duration of the disease varied from 1 to 38 years (mean 11 years) and Raynaud's phenomenon and acrosclerosis were constant features in all the patients. The extent of systemic involvement was assessed using the criteria shown in Table 1 and the resulting 'Disease score' for each patient was then used to examine the correlation between absolute T lymphocyte counts and the severity of the disease.

Controls consisted of forty-five healthy volunteers, fifteen of whom were male, with a mean age of 43·7 ± 14·2 years.

TABLE 1. Criteria of systemic involvement in patients with PSS

System involved	Criteria	Disease score (points allotted for involvement)
Skin	Sclerosis—face	1
	hands	1
	trunk	1
GI tract	Radiological changes	3
Lungs	Radiological changes and/or abnormal CO transfer factor	3
Heart	ECG changes	3
Kidneys	Creatinine clearance < 60 ml/min and/or proteinuria	3
Other	Sjögren's syndrome, myositis, etc.	3

Lymphocytes were obtained from 20 ml of sterile defibrinated venous blood which was always taken between 10 and 11 a.m. to avoid the effect of diurnal variation in the numbers of circulating lymphocytes (Tavadia *et al.*, 1975). After a preliminary sedimentation of red blood cells with 3% gelatin (Plasmagel Laborataire Roger Bellon, Neuilly, France), the residual leukocyte-rich serum was layered over 'Ficol-Triosil' (S.G. 1·077) and centrifuged at 1000 *g* for 20 min (Evans & Middleton, 1970). The cells harvested from the interface were incubated on a cottonwool column for 15 min at 37°C to remove contaminating monocytes and residual polymorphonuclear leukocytes. The purified lymphocyte suspension eluted from the column was washed 3 times with RPMI 1640 (Flow Laboratories, Irvine) and adjusted to a final concentration of 1×10^6 cells/ml in fetal calf serum (Burroughs Wellcome, Beckenham) which had been absorbed with washed sheep red blood cells (SRBC).

Estimation of T lymphocytes

SRBC, used within 7 days of bleeding, were washed 3 times with RPMI 1640 and finally adjusted to a 0·5% suspension. Lymphocytes (5×10^5) were added to duplicate 0·5 ml aliquots of the SRBC suspension, incubated at room temperature for 10 min, centrifuged at 100 *g* for 5 min and then incubated at 4°C for 3 h in an atmosphere of 5% CO_2–95% air. To facilitate the counting of lymphocytes, one drop of 1% toluidine blue was added and the cell pellet was then resuspended by rotation in a Matburn mixer (28 revs/min) for 30 s. Two hundred lymphocytes from each of the duplicate tubes were counted by two observers and the percentage of lymphocytes forming rosettes with 3 or more SRBC was calculated from the mean counts. The absolute T lymphocyte count was then calculated from a differential white blood cell (WBC) count performed on a sequestrene sample taken simultaneously.

Reproducibility

The accuracy of differential WBC counts, as determined by counts on triplicate samples on twenty-four of the controls, was ±5%. The agreement in T lymphocyte counts on duplicate specimens by two observers was, similarly, ±5%.

Statistical methods

The significance of the correlation coefficient for the variation in T lymphocyte numbers with severity of disease in patients with PSS and the differences between total and T lymphocyte numbers in patients and controls were assessed using Student's 't'-test.

RESULTS

The results for absolute total and T lymphocyte counts for patients with PSS and the control group are shown in Table 2. Patients with PSS had both lower total ($t = 3·73$: $P<0·001$) and T lymphocyte ($t = 4·49$: $P<0·001$) counts when compared with controls and this reduction in T lymphocytes showed a correlation ($r = -0·65$: $t = 4·26$: $P<0·001$) with the extent of visceral involvement by the disease (Fig. 1). Thus, when patients were grouped according to the severity of the disease (Table 3), those with only minimal lesions limited to the skin and, perhaps, one major system, had absolute T lymphocyte numbers that were only slightly lower than normal control values ($t = 1·15$: $P>0·1$). By contrast, patients with more extensive visceral involvement had a greater reduction in T lymphocytes ($t = 2·95$: $P<0·01$) which became very marked in those with the most severe disease ($t = 4·40$: $P<0·001$).

TABLE 2. Total and T lymphocyte numbers in patients with PSS and controls

Group	No.	Age (years) (mean ± s.d.)	Total lymphocytes/mm^3 (mean ± s.d.)	T-lymphocytes/mm^3 (mean ± s.d.)	P*
PSS	27	50·5 ± 13·5	1692 ± 615	1149 ± 415	<0·001
Control	45	43·7 ± 14·2	2334 ± 753	1726 ± 583	—

* The P value refers to both total and T lymphocyte figures.

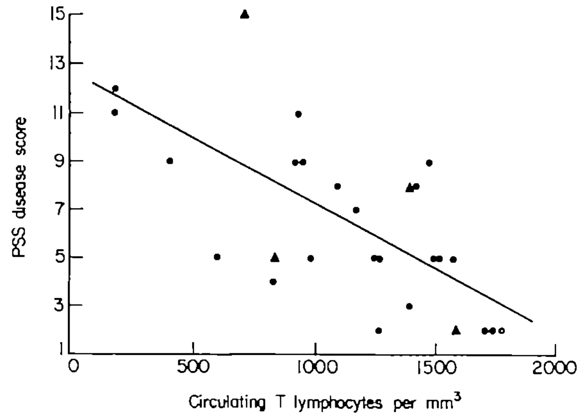


FIGURE 1. The relationship between circulating T lymphocytes and the severity of disease in patients with PSS. ▲ Patients receiving treatment with prednisolone. $r = -0·65$; $t = 4·26$; $P<0·001$.

TABLE 3. T lymphocyte numbers related to disease severity in patients with PSS

PSS group	No.	Age (years) (mean ± s.d.)	ESR (mm/h) (mean ± s.d.)	T lymphocytes/mm^3 (mean ± s.d.)	P*
Mild (disease score 0–4)	7	46·1 ± 17·4	18·1 ± 11·1	1466 ± 399	n.s.
Moderate (disease score 5–8)	12	52·2 ± 11·0	32·5 ± 19·9	1211 ± 294	<0·01
Severe (disease score 9–15)	8	51·7 ± 14·2	51·6 ± 41·9	780 ± 378	<0·001

* Statistical comparison was made with the 45 normal controls. The *P* value refers to T lymphocyte figures only.

DISCUSSION

The present investigation has revealed that patients with PSS have reduced numbers of circulating T lymphocytes. This T-cell deficiency was marked, however, only in those patients with extensive visceral involvement when it could be severe enough to result in an overall lymphopenia. These findings proved to be consistent and reproducible in individual patients when serial estimations were made over an 8–15 month period. By contrast, the T lymphocyte counts of patients with less extensive disease, especially when confined solely to the skin, often fell within the normal control range.

In considering the origin of this deficiency of circulating T cells one must take into account the striking correlation between absolute numbers of T lymphocytes and the extent of visceral involvement by the disease. It is possible that our findings result from a redistribution of lymphocyte subpopulations in PSS rather than a true deficiency, due to a sequestration of sensitized lymphocytes in target organs. The absence of *extensive* lymphocytic infiltrations in the visceral or cutaneous lesions of PSS, however, argues against such an explanation (Rodnan, 1965). Alternatively, the low T cell counts may be indicative of a more general deterioration in cell mediated immunity. Although such a deterioration might be merely the result of advanced disease, it is also possible that the decline in T cell numbers is of aetiological importance. Thus, a decline in functioning 'suppressor' T cells, occurring as part of the wider T-cell deficiency, might be responsible for the emergence of autoimmune phenomena (Allison, Denman & Barnes, 1971) such as autoantibodies and sensitized cytotoxic lymphocytes.

There is evidence from experimental host-versus-graft disease that chronic antigenic stimulation of the host leads to a marked T-cell depletion and that the associated loss of putative 'suppressor' T cells is involved in the hyperglobulinaemia and immune complex deposition of this model (Hard, 1975). PSS is also a disease characterized by hyperglobulinaemia (Šťáva, 1958), autoantibodies (Beck *et al.*, 1963) and sensitized auto-reactive lymphocytes (Hughes *et al.*, 1974; Currie *et al.*, 1971) and the presence of a marked depletion of circulating T cells in those patients with extreme visceral involvement provides further support for the view that auto-aggressive processes are involved in the pathogenesis.

ACKNOWLEDGMENTS

We wish to thank the Department of Haematology, The General Infirmary at Leeds, for assistance with differential white blood cell counts, the Leeds Area Health Authority for financial assistance and all those, both patients and controls, who willingly gave blood for the project.

REFERENCES

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971) Co-operating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet*, **ii**, 135.

BECK, J.S., ANDERSON, J.R., GRAY, K.G. & ROWELL, N.R. (1963) Antinuclear and precipitating autoantibodies in progressive systemic sclerosis. *Lancet*, **ii**, 1188.

CURRIE, S., SAUNDERS, M. & KNOWLES, M. (1971) Immunological aspects of systemic sclerosis: *in vitro* activity of lymphocytes from patients with the disorder. *British Journal of Dermatology*, **84**, 400.

EVANS, C.A. & MIDDLETON, V.L. (1970) Evaluation of methods for preparing pure lymphocyte suspensions from peripheral blood. *Journal of Clinical Pathology*, **23**, 822.

HARD, R.C. JR (1975) Hyperimmunoglobulinaemia, T-cell deficiency and plasmacytosis in RFM mice with host versus graft disease induced by perinatal inoculations of (T6 × RFM) F1 spleen cells. *Clinical and Experimental Immunology*, **22**, 330.

HUGHES, P., HOLT, S. & ROWELL, N.R. (1974) Leukocyte migration inhibition in progressive systemic sclerosis. *British Journal of Dermatology*, **91**, 1.

RODNAN, G.P. (1965) Progressive systemic sclerosis. In: *Immunological Diseases* (Ed. by M. Samter), p. 769. Churchill, London.

ŠŤÁVA, Z. (1958) Serum proteins in scleroderma. *Dermatologica*, **117**, 147.

TAVADIA, H.B., FLEMING, K.A., HUME, P.D. & SIMPSON, H.W. (1975) Circadian rhythmicity of human plasma cortisol and PHA-induced lymphocyte transformation. *Clinical and Experimental Immunology*, **22**, 190.

British Journal of Dermatology (1976) 95, 475.

Macrophage inhibitor factor (MIF) in cutaneous lymphoproliferative diseases

PABLO UMBERT*, RICHARD W. BELCHER AND R.K.WINKELMANN

Mayo Clinic and Mayo Foundation Rochester, Minnesota 55901, U.S.A.

Accepted for publication 12 April 1976

SUMMARY

Macrophage migration inhibitor factor (MIF) activity in the sera of patients with mycosis fungoides, Sézary syndrome, and cutaneous lymphoma was observed in the sera of eight of the ten patients with stage II (infiltrative) mycosis fungoides, but in only one of the eight patients with stage I and in neither of the two patients with stage III mycosis fungoides. Two of the three patients with Sézary syndrome had MIF in the serum. No MIF was observed in cutaneous lymphoma. These data support the concept that Sézary syndrome and mycosis fungoides are T-cell diseases, and transitional, prelymphomatous diseases.

Among the lymphokines postulated to be related to delayed hypersensitivity *in vivo* is the macrophage inhibitor factor (MIF). The *in vitro* detection of MIF-like activity correlates with the state of *in vivo* cellular immunity in man (Rocklin, Meyers & David, 1970a; Rocklin, Rosen & David, 1970b). MIF-like activity has been detected in long-term cultures of various cell types (Papageorgiou & Glade, 1974) and in non-lymphoid cells infected by viruses (Poste, 1975). Many of these factors have physicochemical features and enzyme inactivation properties in common with classical MIF.

MIF-like activity has been demonstrated in the sera of animals with delayed hypersensitivity reaction (Salvin, Youngner & Lederer, 1973), in peritoneal exudate of immunized guinea-pigs (Yoshida & Cohen, 1974a), and recently in the sera of some patients with lymphoproliferative diseases (Cohen *et al.*, 1974; Yoshida *et al.*, 1975). After the intravenous injection of antigen, a depression of monocytes occurs, corresponding to the highest level of MIF activity, and it has been demonstrated that intravenous MIF can suppress delayed hypersensitivity skin reactions in animals (Yoshida & Cohen, 1974b). Yoshida & Cohen (1974b) postulated that these alterations of monocyte numbers were caused by the systemic release of MIF.

Some of the cutaneous lymphoreticular proliferations occur with a background of delayed hypersensitivity and T-cell proliferation. Sézary syndrome (Brouet, Flandrin & Seligmann, 1973; Winkelmann, 1973), parapsoriasis, and mycosis fungoides (Edelson *et al.*, 1974b; Robinowitz, Noguchi &

---

* Research Fellow from the University of Barcelona, Spain.

Reprint requests to: Dr P. Umbert, c/o Section of Publications, Mayo Clinic, 200 First Street SW, Rochester, MN 55901, U.S.A.

Roenigk, 1975) are examples of T-cell proliferation that may develop into lymphoma (Degos *et al.*, 1965). As a consequence of preferential T-cell proliferation, lymphokines may appear in the tissues and circulation. Accordingly, we studied a group of such patients and a group of healthy controls.

MATERIALS AND METHODS

Sera. Ten ml of blood were collected from each patient and from healthy volunteers or patients with other cutaneous disorders who served as controls.

Blood was centrifuged at 3000 rev/min for 30 min to separate the serum. The serum was heat inactivated (56°C for 30 min) and stored at −70°C in a sterile glass tube until the day of assay.

Peritoneal exudate cells from a normal female guinea-pig weighing 450 g were induced with light mineral oil and collected as previously described by Bloom & Glade (1971). The cells (1×10^6, 90% macrophages) were washed in 70–100 ml of cold TCC 199 medium and suspended to 10% by volume in TCC 199 containing 15% normal heat-inactivated guinea-pig serum (Remold *et al.*, 1970).

Capillary tubes were filled with the cell suspension, sealed with wax, and centrifuged. The tubes were cut, and the portion containing the cells was placed in Mackaness chambers.

Macrophage migration from those capillaries was assayed in a medium containing 50% serum from patients to be tested and 50% of TCC 199 with 50/μg/ml streptomycin and 50 U/ml penicillin. Macrophage migration in a medium containing equal volumes of sera from normal subjects and TCC 199 with Penistrept served as control.

The chambers were incubated for 24 h at 37°C in a humidified 5% CO_2–95% air incubator, and the area of migration of cells from the capillary tubes was measured by planimetry:

$$\%\ \text{inhibition} = 100 \times \frac{\text{average area of migration in MIF serum of patient}}{\text{average area of migration in MIF of serum of control}}.$$

The reversibility of the phenomenon was demonstrated in three assays when the culture was maintained for several days.

For each serum sample, a minimum of three chambers was done. In all these assays, the MIF activity satisfied the usual criteria for such activity (Bloom & Glade, 1971), namely the absence of cytotoxicity as measured by trypan blue exclusion (more than 80% viability). Cutaneous delayed hypersensitivity tests were done with 0·1 ml of the following tests: *Candida albicans* (Hollister-Stier Laboratories, 1 to 100 concentration), *Trichophyton* (Hollister-Stier Laboratories, 1 to 30 concentration), streptokinase-streptodornase (Lederle Laboratories, 1 to 100 concentration), purified protein derivative (Connaught Laboratories, 0·001 mg concentration), histoplasmin (Parke, Davis and Company, 0·01 ml concentration), mumps (Lilly and Company), and control. An indurated reaction of 10 mm or greater to one of the antigens was considered a positive reaction.

Statistics. Cases with their control value were chosen so that each pair of observations was independent; that is, no control or case was used more than once. In accordance with the usual criteria (Cohen *et al.*, 1974), a migration index of 80 was taken as significant inhibition; an index of 100 represents no MIF.

GROUP OF PATIENTS

Twenty patients had mycosis fungoides. These were classified according to the criteria of Lever & Schaumburg-Lever (1975), Van Scott & Haynes (1971), and Samman (1975): stage I, polymorphous parapsoriasis, eight patients; stage II, infiltrative lesions, ten patients; and stage III, tumours, two patients. Five patients had primary cutaneous lymphomas. This category indicates that all studies failed to reveal systemic disease (including X-rays, bone marrow, liver and spleen scans, and lymphangiogram). Nine patients had other cutaneous diseases: bullous pemphigoid, bullous erythema multiforme, psoriasis, keratoderma of palms and soles, pemphigus, pityriasis rosea, elastoidosis, vasculitis, and acute streptococcal infection. Three patients had Sézary syndrome. A healthy control was matched for each patient.

All patients with stages I and II mycosis fungoides were receiving topical treatment only, and none had had radiotherapy for a minimum of 3 months before study. In patients with stage III mycosis fungoides, primary cutaneous lymphoma, or Sézary syndrome, the serum was obtained before the treatment, with the exception of one patient with Sézary syndrome, who was undergoing repeated leukapheresis (Edelson *et al.*, 1974a).

RESULTS

In our patients with mycosis fungoides, there were significant differences from controls in serum MIF activity in relation to the stages of the disease (Table 1 and Fig. 1). In stage I mycosis fungoides, no lymphokines were demonstrated, and only one patient had MIF-like activity in his serum. In contrast, in stage II, which corresponds to the infiltrative lesions, there was significant MIF activity in the sera of eight of ten patients. Lymphokines were not detected in the sera of patients with stage III mycosis fungoides or in patients with primary cutaneous lymphoma. Two patients with Sézary syndrome demonstrated serum MIF-like activity. The only patient with Sézary syndrome who had no MIF had had repeated leukapheresis treatment which had reduced the T-cell population in the peripheral blood.

TABLE 1. Serum MIF activity in patients with cutaneous lymphoproliferative diseases

Disease	No. of patients	MIF activity Mean	MIF activity s.d.
Mycosis fungoides			
Stage I	8	93·3‡	17·2
Stage II	10	74·8*	10·2
Stage III	2	83·1	2·8
Cutaneous lymphoma	5	97·1†	19·3
Sézary syndrome	3	79·9	24·0
Other cutaneous diseases	9	99·8§	8·7

Significantly different from controls: * $P<0·07$; † $P<0·06$; ‡ $P<0·03$; and § $P<0·01$.

The five patients with stage I mycosis fungoides who were skin-tested had one or more positive reactions to the delayed cutaneous hypersensitivity test (Table 2). In stage II mycosis fungoides, eight patients were tested (seven with circulating activity MIF), and four had negative reactions. The reaction was positive in both patients with stage III disease. All three patients with Sézary syndrome had negative reactions. Of the two patients with primary cutaneous lymphomas, one had a positive reaction and the other negative.

There was no significant relationship between the presence of MIF activity and delayed hyper-

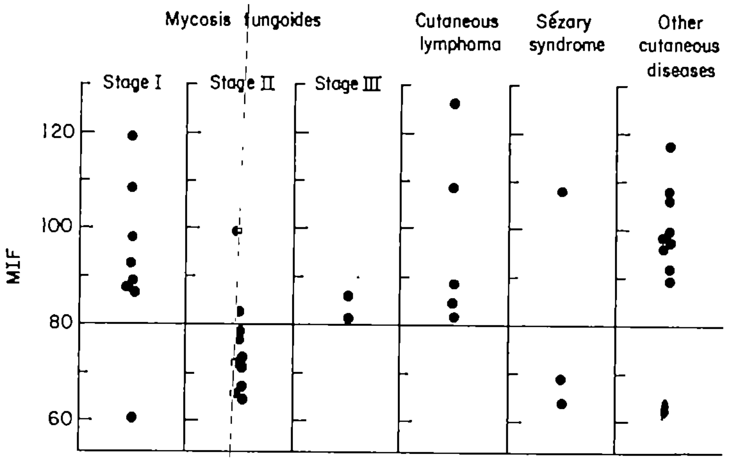


FIGURE 1. Serum MIF activity in patients with cutaneous lymphoproliferative disease. A migration index of 80 or below represents significant inhibition.

sensitivity. Sixty-seven percent of our patients with MIF activity had no delayed hypersensitivity demonstrated. Seventy percent with no MIF activity did have positive delayed hypersensitivity skin tests. There was no significant correlation between the presence of MIF activity and the percentage of monocytes in the peripheral blood.

DISCUSSION

Lymphokine production represents a general biological phenomenon as well as being part of the effector mechanisms for cell-mediated immunity. Among the mediators produced by highly purified human T- and B-lymphocytes is MIF (Rocklin *et al.*, 1974; Wahl *et al.*, 1975), and the T-cells producing MIF are proliferating, whereas B-cells producing this mediator are not (Rocklin *et al.*, 1974). Intradermal injection of activated lymphocyte supernatants induces indurated skin lesions similar to those of delayed hypersensitivity reaction (Yoshida, Nagai & Hashimoto, 1973).

TABLE 2. Serum MIF cutaneous delayed hypersensitivity in various diseases

Disease	No. of patients	Delayed hypersensitivity		MIF activity	
		Pos.	Neg.	Yes	No
Mycosis fungoides					
Stage I	5	5	0	0	5
Stage II	8	4	4	7	1
Stage III	2	2	0	0	2
Cutaneous lymphoma	2	1	1	0	2
Sézary syndrome	3	0	3	2	1

In 1974, Yoshida & Cohen (1974a) detected MIF-like activity in the sera of patients with such lymphoproliferative diseases as chronic lymphocytic leukaemia, Hodgkin's disease, and lymphoma (Cohen *et al.*, 1974). In 1975, Yoshida *et al.* observed that the abnormal lymphocytes from five patients with

Sézary syndrome produce MIF-like activity both *in vitro* and *in vivo,* and there was a suggestive negative relationship between serum MIF activity and the ability of lymphocytes to release MIF into culture medium; that is, the cells from patients with the greatest serum activity resulted in culture preparations entirely devoid of MIF.

Our studies confirm the MIF activity in the sera of patients with Sézary syndrome; in one patient, leukapheresis not only reduced the T-cell population but also may have removed MIF activity, which could explain its benefit in patients with Sézary syndrome (Edelson *et al.*, 1974a). Our results indicate a change in mycosis fungoides, with the peak MIF activity in stage II and a disappearance in the later stages which was related to the evolution of the disease or to the inflammation associated with it. Furthermore, in stage II disease, four of the seven patients with MIF activity had no delayed hypersensitivity in contrast to patients with stage I or III disease who had delayed hypersensitivity, suggesting a relationship similar to that observed in animals between MIF and delayed hypersensitivity. More cases observed in all stages of mycosis fungoides will be needed to evaluate this for lymphoproliferative diseases in man. Two of the three patients with Sézary syndrome had lymphokines in the sera and also had no delayed hypersensitivity. In Hodgkin's disease, the delayed hypersensitivity test is known to be reversible with treatment and is related to the prognosis of the disease (Aisenberg, 1971). This may account for the positive delayed hypersensitivity in our patients with stage III mycosis fungoides, or it may indicate that the patients with stage III disease had a unique course.

Our findings and those of Yoshida & Cohen (1974a, b) do not provide information about the cellular sources of MIF-like activity observed, the nature of stimulus for the production of the factors involved, or the degree of specificity of this activity for the diseases studied. Nevertheless, since the Sézary syndrome is characterized by T cells principally in the skin (Brouet *et al.*, 1973; Edelson *et al.*, 1974b), the source of the chemical mediators may be the skin through contact with the possible antigen. Mycosis fungoides also is considered a T-cell disease of the skin (Brouet *et al.*, 1973; Robinowitz *et al.*, 1975). Because T cells produce various lymphokines, including MIF, increased amounts of circulating lymphokines might be expected in extensive proliferative T-cell disease. A quantitative study of T and B cells in mycosis fungoides revealed no relationship between the numbers of circulating T and B cells and the presence or absence of MIF. Because no MIF activity is found in stage I mycosis fungoides, T-cell exposure in the skin may not be enough by itself to cause measurable circulating MIF activity. Circulating endogenous MIF activity may exert an inhibitory effect on the skin activity.

It is of major interest that two lymphoproliferative diseases of the skin, mycosis fungoides and Sézary syndrome, which may be transitional states between inflammation and lymphoma, are associated with a circulating lymphokine, MIF activity. More data on the proliferative phase of these important skin diseases may provide the immunological knowledge necessary to control them.

REFERENCES

AISENBERG, A.C. (1971) Hodgkin's disease. In: *Immunological Disease* (Ed. by M.Samter), 2nd edition, p. 571. Little, Brown & Co., Boston.

BLOOM, B.R. & GLADE, P.R. (1971) In vitro *Methods in Cell-Mediated Immunity*. Academic Press, New York.

BROUET, J.C., FLANDRIN, G. & SELIGMANN, M. (1973) Indication of thymus-derived nature of proliferating cells in 6 patients with Sézary syndrome. *New England Journal of Medicine*, **289**, 341.

COHEN, S., FISHER, B., YOSHIDA, T. & BETTIGOLE, R.E. (1974) Serum migration-inhibitory activity in patients with lymphoproliferative diseases. *New England Journal of Medicine*, **290**, 882.

DEGOS, R., CIVATTE, J., TOURAINE, R., OSSIPOVSKI, B. & GONZALEZ-MENDOZA, A. (1965) Confrontation anatomo-clinique de 129 hémoréticulopathies malignes cutanées. *Annales de Dermatologie et Syphiligraphie*, **92**, 121.

EDELSON, R., FACKTOR, M., ANDREWS, A., LUTZNER, M. & SCHEIN, P. (1974a) Successful management of Sézary syndrome: mobilization and removal of extravascular neoplastic-T cells by leukapheresis. *New England Journal of Medicine*, **291**, 293.

EDELSON, R.L., KIRKPATRICK, C.H., SHEVACH, E.M., SCHEIN, P.S., SMITH, R.W., GREEN, I. & LUTZNER, M. (1974b) Preferential cutaneous infiltration by neoplastic thymus-derived lymphocytes: morphologic and functional studies. *Annals of Internal Medicine*, **80**, 685.

LEVER, W.F. & SCHAUMBURG-LEVER, G. (1975) *Histopathology of the Skin*, 5th edition. J.B.Lippincott Company, Philadelphia.

PAPAGEORGIOU, P.S. & GLADE, P.R. (1974) Migration inhibitory factor (MIF) production by skin fibroblast cultures from patients with severe combined immunodeficiency. *Cellular Immunology*, **12**, 326.

POSTE, G. (1975) Production of macrophage migration inhibitory factor(s) (MIF) virus-transformed cells. *Experimental Cell Research*, **92**, 283.

REMOLD, H.G., KATZ, A.B., HABER, E. & DAVID, J.R. (1970) Studies on migration inhibitory factor (MIF): recovery of MIF activity after purification by gel filtration and disc electrophoresis. *Cellular Immunology*, **1**, 133.

ROBINOWITZ, B.N., NOGUCHI, S. & ROENIGK, H.H., JR (1975) Tumor cell characterization in mycosis fungoides (abstract). *Journal of Investigative Dermatology*, **64**, 286.

ROCKLIN, R.E., MACDERMOTT, R.P., CHESS, L., SCHLOSSMAN, S.F. & DAVID, J.R. (1974) Studies on mediator production by highly purified human T and B lymphocytes. *Journal of Experimental Medicine*, **140**, 1303.

ROCKLIN, R.E., MEYERS, O.L. & DAVID, J.R. (1970a) An *in vitro* assay for cellular hypersensitivity in man. *Journal of Immunology*, **104**, 95.

ROCKLIN, R.E., ROSEN, F.S. & DAVID, J.R. (1970b) *In vitro* lymphocyte response of patients with immunologic deficiency diseases: correlation of production of macrophage inhibitory factor with delayed hypersensitivity. *New England Journal of Medicine*, **282**, 1340.

SALVIN, S.B., YOUNGNER, J.S. & LEDERER, W.H. (1973) Migration inhibitory factor and interferon in circulation of mice with delayed-hypersensitivity. *Infection and Immunity*, **7**, 68.

SAMMAN, P.D. (1975) Cutaneous reticuloses. *Transactions of St John's Hospital Dermatological Society*, **61**, 11.

VAN SCOTT, E.J. & HAYNES, H.A. (1971) Cutaneous lymphomas. In: *Dermatology in General Medicine* (Ed. by T.B.Fitzpatrick, K.A.Arndt, W.H.Clark, Jr, A.Z.Eisen, E.J.Van Scott & J.H.Vaughan), p. 556. McGraw-Hill, New York.

WAHL, S.M., WILTON, J.M., ROSENSTREICH, D.L. & OPPENHEIM, J.J. (1975) Role of macrophages in the production of lymphokines by T-lymphocytes and B-lymphocytes. *Journal of Immunology*, **114**, 1296.

WINKELMANN, R.K. (1973) T cell erythroderma (Sézary syndrome). *Archives of Dermatology*, **108**, 205.

YOSHIDA, T. & COHEN, S. (1974a) *In vivo* manifestations of lymphokine and lymphokine-like activity. In: *Mechanism of Cell-Mediated Immunity* (Ed. by R.T.McCluskey & S.Cohen), p. 43. John Wiley, New York.

YOSHIDA, T. & COHEN, S. (1974b) Lymphokine activity in vivo in relation to circulating monocyte levels and delayed skin reactivity. *Journal of Immunology*, **112**, 1540.

YOSHIDA, T., EDELSON, R., COHEN, S. & GREEN, I. (1975) Migration inhibitory activity in serum and cell supernatants in patients with Sézary syndrome. *Journal of Immunology*, **114**, 915.

YOSHIDA, T., NAGAI, R. & HASHIMOTO, T. (1973) Lack of species specificity of a skin-reactive factor released from sensitized guinea-pig and spleen cells. *Laboratory Investigation*, **29**, 329.

British Journal of Dermatology (1976) **95**, 481.

Lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare

PABLO UMBERT*, RICHARD W. BELCHER AND R.K. WINKELMANN

The Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901, U.S.A.

Accepted for publication 9 April 1976

SUMMARY

Serum MIF activity was studied in ten patients with sarcoidosis, fourteen with granuloma annulare, four with necrobiosis lipoidica, and nine with various dermatological diseases. Positive MIF activity was found in the sera of nine of the ten patients with sarcoidosis and eleven of the fourteen patients with granuloma annulare. The delayed hypersensitivity tests were negative in all nine of the patients with sarcoidosis who had serum MIF activity and were positive in only three patients with cutaneous sarcoid lesions. One of four patients with necrobiosis lipoidica demonstrated minimal serum MIF activity. Data on serum lymphokine activity in sarcoidosis and granuloma annulare suggest that these two diseases are related to delayed hypersensitivity mechanisms.

Serum macrophage inhibitor factor (MIF) is detected in animals during delayed hypersensitivity reaction but never in the serum of unimmunized animals or in control animals that are immunized but unchallenged (Yoshida & Cohen, 1974a). MIF-like activity has been demonstrated in the peritoneal exudates of immunized guinea-pigs (Yoshida & Cohen, 1974b) and recently in the sera of some patients with lymphoproliferative diseases (Cohen *et al.*, 1974; Umbert, Belcher & Winkelmann, 1976).

Lymphocytes from some patients with sarcoidosis show an enhanced spontaneous (non-stimulated) blastogenic response *in vitro* (Buckley, Nagaya & Sieker, 1966). This response occurs particularly after cultivation of the patient's lymphocytes for 5–7 days *in vitro* and may be associated with release of lymphokines. In some lymphoproliferative diseases, enhanced lymphokine (MIF) release also occurs *in vivo* (Cohen *et al.*, 1974), and recently we demonstrated increased serum MIF activity in some patients with mycosis fungoides and Sézary syndrome (Umbert *et al.*, 1976). Because of this altered skin test reactivity in patients with certain granulomatous diseases, we studied patients who had sarcoidosis and granuloma annulare.

---

* Research Fellow from the University of Barcelona, Spain.

Reprint requests to: Dr P.Umbert, c/o Section of Publications, Mayo Clinic, 200 First Street SW, Rochester, MN 55901, U.S.A.

MATERIALS AND METHODS

Sera. Ten milliliters of blood were collected from each patient and from healthy volunteers or patients with other cutaneous disorders who served as controls.

Blood was centrifuged at 3000 rev/min for 30 min to separate the serum. The serum was heat inactivated (56°C for 30 min) and stored at $-70°C$ in sterile glass tubes until the day of assay.

Peritoneal exudate cells from a normal female guinea-pig weighing 450 g were induced with light mineral oil and collected, as previously described by David & David (1971). The cells (1×10^6, 90% macrophages) were washed in 70–100 ml of cold TCC 199 medium and suspended to 10% by volume in TCC 199 containing 15% normal heat-inactivated guinea-pig serum (Remold *et al.*, 1970).

Capillary tubes were filled with the cell suspension, sealed with wax, and centrifuged. The tubes were cut, and the portion containing the cells was placed in Mackaness chambers.

Macrophage migration from those capillaries was assayed in a medium containing 50% serum from patients to be tested and 50% of TCC 199 with 50 μg/ml streptomycin and 50 U/ml of penicillin. Macrophage migration in a medium containing equal volumes of serum from normal subjects and TCC 199 with Penistrept served as control.

The chambers were incubated for 24 h at 37°C in a humidified 5% CO_2 and 95% air incubator, and the area of migration of cells from the capillary tubes was measured by planimetry:

$$\% \text{ inhibition: } 100 \times \frac{\text{average area of migration in MIF serum of patient}}{\text{average area of migration in MIF of serum of control}}.$$

For each serum sample a minimum of three chambers was done. In all assays, the MIF activity satisfied the usual criteria (David & David, 1971); namely, the absence of cytotoxicity was measured by trypan blue exclusion (more than 80% viability). Cutaneous delayed hypersensitivity tests were done with 0·1 ml of the following antigens only in the patients with sarcoidosis: *Candida albicans* (Hollister-Stier Laboratories, 1 to 100 concentration), *Trichophyton* (Hollister-Stier Laboratories, 1 to 30 concentration), streptokinase-streptodornase (Lederle Laboratories, 1 to 100 concentration), purified protein derivative (Connaught Laboratories, 0·001 mg concentration), histoplasmin (Parke, Davis and Company, 0·01 ml concentration), mumps (Lilly & Company), and control (normal saline). An indurated reaction of 10 mm or greater at 48 hours to one of the antigens was considered a positive reaction.

Statistics. Cases with their control value were chosen so that each pair of observations was independent—that is, no control or case was used more than once. When the ratio of MIF case to MIF control was formed, the average of their values was used in the denominator. In accordance with the usual criteria (Cohen *et al.*, 1974), a migration index of 80% was taken as significant inhibition; an index of 100% represents no MIF.

GROUPS OF PATIENTS

Sarcoidosis. Of the ten patients studied, nine had systemic involvement, as determined by pulmonary or lymph node biopsy, and two of the nine also had cutaneous manifestations. The tenth patient had sarcoidosis limited to the skin. Thus, only three patients had cutaneous sarcoidosis.

Granuloma annulare. All fourteen patients had typical clinical and histological findings characteristic of granuloma annulare.

Necrobiosis lipoidica. The four patients presented plaques of localized necrobiosis lipoidica in the lower extremities, but only one had diabetes.

Other cutaneous diseases. These nine patients had the following diagnoses: bullous pemphigoid, bullous erythema multiforme, psoriasis, keratoderma of palms and soles, pemphigus, pityriasis rosea, elastoidosis, vasculitis, and acute streptococcal infection.

RESULTS

Nine of the ten patients with visceral sarcoidosis demonstrated serum MIF-like activity. The average area of % inhibition of macrophages was 65·9 (Table 1 and Fig. 1), well below the control limits of 80 ($P < 0\cdot002$). Of the fourteen patients with granuloma annulare, eleven showed MIF-like activity, with the average area of % inhibition of 68·2 ($P < 0\cdot05$). Only one patient with necrobiosis lipoidica showed minimal MIF-like activity. The few patients do not allow a conclusive statement, but the results in general were negative. The control dermatoses had normal macrophage migration and no serum inhibitory factor.

TABLE 1. Serum MIF activity in patients with cutaneous granulomas

Disease	No. of patients	MIF activity, % inhibition Mean	s.d.
Sarcoidosis	10	65·9*	11·8
Granuloma annulare	14	68·2†	24·0
Necrobiosis lipoidica	4	97·2†	14·0
Other cutaneous diseases	9	99·8‡	8·7

Significantly different from control values: * $P < 0\cdot002$; † $P < 0\cdot05$; ‡ $P < 0\cdot01$.

Purified protein derivative (PPD) at intermediate strength (5 tuberculin units) was used in all but one patient. Only three patients had positive delayed hypersensitivity, and these were the same three

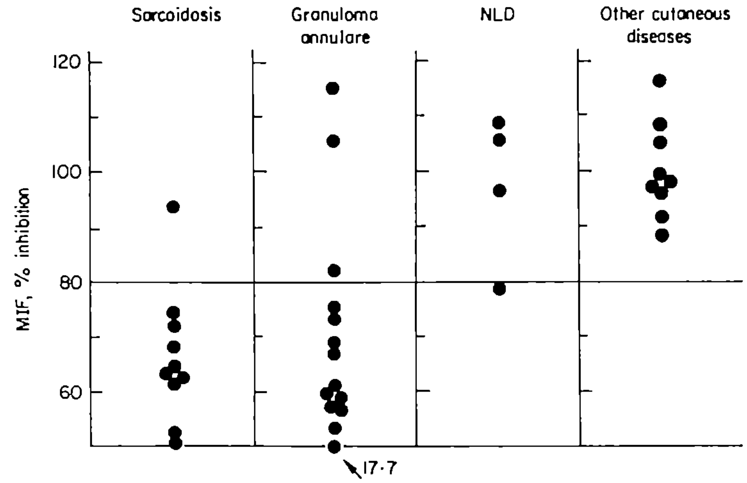


FIGURE 1. Serum MIF activity in patients with cutaneous granulomas. NLD, necrobiosis lipoidica.

who had cutaneous sarcoid. Each of the three was positive to different antigens: one to PPD on two different occasions, one to streptodornase and histoplasmin, and the third to *Trichophyton* and histoplasmin. Of nine patients with systemic involvement, two were receiving alternate-day corticosteroid therapy when the study was done. This treatment did not abolish the positive serum MIF-like activity.

DISCUSSION

Our studies demonstrated MIF-like activity in the sera of patients with visceral sarcoidosis and cutaneous granuloma annulare. The lymphokine production phenomenon may represent a form of immunological host defence or may be induced by non-immunological means. Among the lymphokines postulated to be related to delayed hypersensitivity *in vivo* is MIF. MIF-like activity has been studied extensively, and its detection *in vitro* is used widely as a correlate for the state of cellular immunity in man *in vivo* (Rocklin, Meyers & David, 1970; Rocklin, Rosen & David, 1970). A recent study has shown that, after the intravenous injection of antigen, a depression of monocytes occurred that corresponded to the highest level of MIF activity and with the suppression of delayed hypersensitivity skin reactions in animals (Yoshida & Cohen, 1974a; Sonozaki *et al.*, 1975). Such a reaction might occur in patients with sarcoidosis, in whom the circulating MIF could be related to the abnormality of skin test reactivity.

Several well-documented examples of human hypersensitivity diseases have illustrated the formation of epithelioid cell nodules or masses in the reticuloendothelial system. Such granulomas may be indistinguishable from those of sarcoidosis. Epithelioid granulomas of sarcoidosis might be regarded as a morphological expression of an obscure specific hypersensitivity. Recently, we reported (Umbert & Winkelmann, unpublished data b) an overlap of clinical and histopathological findings between granuloma annulare and sarcoidosis, and we postulated that, in some of the patients, a similar pathogenic mechanism is related to both diseases.

Sarcoidosis is characterized in electron microscopy by the presence of histiocytes, epithelioid cells, and activated macrophages (Jones-Williams *et al.*, 1971). Delayed hypersensitivity involving thymus-derived lymphocytes and their lymphokines seems to be related to an increase in the rate at which young macrophages arrive in the lesion. Recently, we observed in granuloma annulare (Umbert & Winkelmann, unpublished data a) the presence of monocytes, activated macrophages, and dying macrophages, thus indicating the possibly high turnover. In addition, we found localized degeneration of collagen and elastic fibres, which might be related to the collagenase and elastase released from activated macrophages. Blood-borne macrophages may be activated locally where the possible antigens and their products or non-immunological stimuli activate the macrophage via the lymphokine and cytophilic antibodies or other means (Page, Davies & Allison, 1974). Recently, we demonstrated, by immunofluorescent studies of ten patients with granuloma annulare, the absence of leukocytoclastic vasculitis and immune complexes. In contrast, we found the nodular deposition of fibrin outside the vessels in the area of necrobiosis and granuloma in all of our patients with granuloma annulare and sarcoidosis (Umbert & Winkelmann, 1976). Previous reports emphasized the observation of fibrin in delayed hypersensitivity reactions of experimental type and clinical type such as the Kveim test (Salo & Hannuksela, 1972).

Our present MIF results indicate that immediate hypersensitivity is not involved in the production of these granulomas but that delayed hypersensitivity may be. All three patients who had cutaneous sarcoidosis with or without visceral involvement had positive cutaneous delayed hypersensitivity. In contrast, all six patients who had systemic sarcoidosis without cutaneous involvement had negative reactions to PPD. This difference may explain why negative delayed hypersensitivity has not been documented in granuloma annulare. These studies raise the question of the role of lymphokines in diseases characterized by granulomatous inflammation. Indeed, mononuclear phagocytes have been activated by lymphokines *in vitro*. Further studies of granulomatous processes are indicated to determine the mechanisms of lymphokine production and the part that these mediators may have in the pathogenesis of these diseases.

ACKNOWLEDGMENTS

Thanks are due to Dr R.A.DeRemee, Division of Thoracic Diseases and Internal Medicine, and Dr J.D.Stobo, Department of Immunology, Mayo Clinic, Rochester, Minnesota, who provided serum samples for this study.

REFERENCES

BUCKLEY, C.E., III, NAGAYA, H. & SIEKER, H.O. (1966) Altered immunologic activity in sarcoidosis. *Annals of Internal Medicine*, **64**, 508.

COHEN, S., FISHER, B., YOSHIDA, T. & BETTIGOLE, R.E. (1974) Serum migration-inhibitory activity in patients with lymphoproliferative diseases. *New England Journal of Medicine*, **290**, 882.

DAVID, J.R. & DAVID, R. (1971) Assay for inhibition of macrophage migration. In: *In Vitro Methods in Cell-Mediated Immunity* (Ed. by B.R.Bloom & P.R.Glade), p. 249. Academic Press, New York.

JONES-WILLIAMS, W., ERASMUS, D.A., JENKINS, D., JAMES, E.M. & DAVIES, T. (1971) A comparative study of the ultrastructure and histochemistry of sarcoid and tuberculous granulomas. In: *Fifth International Conference on Sarcoidosis* (Ed. by L.Levinsky & F.Macholda), p. 115. Universita Karlova, Praha.

PAGE, R.C., DAVIES, P. & ALLISON, A.C. (1974) Participation of mononuclear phagocytes in chronic inflammatory diseases. *Journal of the Reticuloendothelial Society*, **15**, 413.

REMOLD, H.G., KATZ, A.B., HABER, E. & DAVID, J.R. (1970) Studies on migration inhibitory factor (MIF): recovery of MIF activity after purification by gel filtration and disc electrophoresis. *Cellular Immunology*, **1**, 133.

ROCKLIN, R.E., MEYERS, O.L. & DAVID, J.R. (1970) An *in vitro* assay for cellular hypersensitivity in man. *Journal of Immunology*, **104**, 95.

ROCKLIN, R.E., ROSEN, F.S. & DAVID, J.R. (1970) *In vitro* lymphocyte response of patients with immunologic deficiency diseases: correlation of production of macrophage inhibitory factor with delayed hypersensitivity. *New England Journal of Medicine*, **282**, 1340.

SALO, O.P. & HANNUKSELA, M. (1972) Immunohistology of the Kveim reaction. *Annals of Clinical Research*, **4**, 169.

SONOZAKI, H., PAPERMASTER, V., YOSHIDA, T. & COHEN, S. (1975) Desensitization: effects on cutaneous and peritoneal manifestations of delayed hypersensitivity in relation to lymphokine production. *Journal of Immunology*, **115**, 1657.

UMBERT, P., BELCHER, R.W. & WINKELMANN, R.K. (1976) Macrophage inhibitor factor (MIF) in cutaneous lymphoproliferative diseases. *British Journal of Dermatology*, **95**, 475.

UMBERT, P., & WINKELMANN, R.K. (1976) Granuloma annulare: direct immunofluorescent study. *British Journal of Dermatology*, **95**, 487.

UMBERT, P. & WINKELMANN, R.K. Histologic, histochemical and ultrastructural studies of granuloma annulare (unpublished data a).

UMBERT, P. & WINKELMANN, R.K. Sarcoidosis and granuloma annulare (unpublished data b).

YOSHIDA, T. & COHEN, S. (1974a) Lymphokine activity *in vivo* in relation to circulating monocyte levels and delayed skin reactivity. *Journal of Immunology*, **112**, 1540.

YOSHIDA, T. & COHEN, S. (1974b) *In vivo* manifestations of lymphokine and lymphokine-like activity. In: *Mechanisms of Cell-Mediated Immunity* (Ed. by R.T.McCluskey & S.Cohen), p. 43. John Wiley, New York.

British Journal of Dermatology (1976) **95**, 487.

Granuloma annulare: direct immunofluorescence study

PABLO UMBERT* AND R.K.WINKELMANN

The Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901, U.S.A.

Accepted for publication 9 April 1976

SUMMARY

Direct immunofluorescence studies were carried out in eleven specimens of granuloma annulare. The results (in all cases) indicate the presence of focal deposition of fibrin, localized primarily in the intervascular portion of the dermis, corresponding to the granulomatous and necrobiotic areas. These findings implicate the cellular mechanism of delayed hypersensitivity with focal involvement of the clotting system in the development of granuloma annulare lesion.

Granuloma annulare is a benign, chronic skin disease of unknown cause characterized clinically by dermal papules and nodules, usually in annular configuration. Microscopically, the disease presents foci of necrobiotic collagen and mucinosis surrounded by mononuclear cells, often in a pallisade arrangement (Civatte, 1967). In most cases (Umbert & Winkelmann, unpublished data), histological study reveals a characteristic infiltration of the dermal collagen by mononuclear cells. Occasionally, a more granulomatous reaction is seen with an epithelioid and giant-cell component simulating the histological features of sarcoidosis (Umbert & Winkelmann, unpublished data).

Although the cause of granuloma annulare is unknown, a number of possibilities have been suggested: insect bites (Moyer, 1964), sunlight (Tolmach, 1961; Dicken, Carrington & Winkelmann, 1969; Leppard & Black, 1972), diabetes mellitus (Hammond, Dyess & Castro, 1972; Haim *et al.*, 1973), and after tuberculin testing (Beer & Wilson Jones, 1966). Whereas in the early portion of this century granuloma annulare was considered to be a tuberculid, this relationship could not be substantiated and has been discarded. We believe that granuloma annulare occurs through an immune reaction to unknown antigens. Therefore, we undertook an immunological study of granuloma annulare, and this report shows our findings in direct immunofluorescence studies of eleven specimens of granuloma annulare, three of necrobiosis lipoidica, and one of cutaneous sarcoidosis. The results indicate that massive, focal fibrin deposition is consistently found in granuloma annulare and suggest a cellular mechanism of delayed hypersensitivity with focal involvement of the clotting system.

---

* Research Fellow from the University of Barcelona, Spain.

Reprint requests to: Dr P. Umbert, c/o Section of Publications, Mayo Clinic, 200 First Street SW, Rochester, MN 55901, U.S.A.

METHOD

Patient population. All eleven specimens from ten patients had classical granuloma annulare, clinically and histologically. The biopsy specimens for immunofluorescence (IF) study were taken from the border of the lesion where there was more clinical activity.

Specimen. A punch biopsy (3 mm) was done in each case, and the specimen was frozen immediately in liquid nitrogen and stored at −70°C until used.

Antisera. Monospecific antisera IgG were prepared, assayed, and conjugated with fluorescein isothiocyanate according to the method of Triftshauser, Hayden & Beutner (1970). Monospecific antisera to human IgA, IgM, β_{1C}/β_{1A} globulins, and fibrin were purchased from a commercial source* and tested for specificity by both immunodiffusion (Ouchterlony) and immunoelectrophoresis. Ratios of fluorescein to protein, units of antiserum and antibody protein assays, and dilutions at which these conjugated antisera were used conformed to previously determined standards (Triftshauser *et al.*, 1970; Jordon, Triftshauser & Schroeter, 1971).

Immunofluorescence procedures. Direct IF staining of skin biopsy specimens, performed by methods previously outlined (Jordon *et al.*, 1971), was carried out using monospecific conjugated antisera to IgG, IgM, IgA, C3, β_{1C}/β_{1A} globulins, and fibrin. Dilutions used for these antisera varied (Table 1).

TABLE 1. Characteristics of labeled antihuman antisera

Antiserum	F/P ratio*	Unitage†	Use dilution
Anti-IgG	3·2	8	1:32
Anti-IgA	2·5	8	1:32
Anti-IgM	3·0	4	1:32
Anti-C3	3·0	4	1:20
Anti-fibrin	2·2	—	1:16

* Ratio of molar fluorescein to protein. † Units determined in gel diffusion by standard methods (Beutner, Chorzelski & Jordon, 1970).

RESULTS

The results in granuloma annulare (Table 2) showed the consistent presence of a fibrin mass in all eleven specimens. The mass was primarily localized in the extravascular portion of the dermis, which corresponds to the granulomatous and necrobiotic areas (Fig. 1). A major feature was the absence of the fibrin mass in blood vessels; only one specimen showed a vascular deposition of the fibrin mass. Four of the eleven specimens had IgM cytoid bodies in the papillary dermis. IgM and C3 were found in the vessels of one patient. Basement membrane immunofluorescence was noted in three patients: IgM in two and C3 in the other. All three had a very fine granular pattern of deposition. In two patients, we observed IgM deposition in the dermis which had the same morphological appearance as that found for fibrin. We found C3 in three other patients; however, the amount and intensity of the fluorescence was less.

In our one case of cutaneous sarcoidosis (Table 3), we found the same pattern of circumscribed deposition of fibrin mass between collagen fibers corresponding to the site of the non-caseating granuloma.

---

* Hyland Division, Travenol Laboratories, Costa Mesa, CA 92626.

TABLE 2. Results of direct immunofluorescence in ten cases of cutaneous granuloma annulare

Case	Dermal–epidermal junction	Cytoid-like bodies	Superficial vessels	Dermis
1	Neg.	Neg.	Neg.	Fibrin
2	Neg.	Neg.	Fibrin	Fibrin
3a	Neg.	IgM	Neg.	Fibrin
b	IgM, fine granular	IgM	Neg.	Fibrin, IgM
4	IgM, fine granular	IgM	IgM, C3	Fibrin, C3
5	Neg.	IgM	Neg.	Fibrin
6	C3, fine granular	Neg.	Neg.	Fibrin, C3
7	Neg.	Neg.	Neg.	Fibrin, C3
8	Neg.	Neg.	Neg.	Fibrin
9	Neg.	Neg.	Neg.	Fibrin
10	Neg.	Neg.	Neg.	Fibrin, IgM

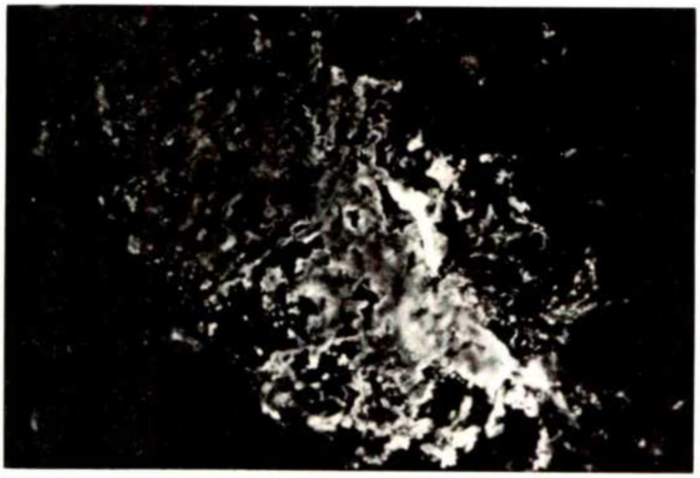


FIGURE 1. Direct IF staining of skin lesions from patient with granuloma annulare. Positive staining with conjugate anti-fibrin (× 80). Localized primarily in extravascular portion of dermis.

Among the three cases of necrobiosis lipoidica, two had deposition of fibrin mass in the dermal vessels and one had a weak deposition of fibrin in the dermis.

DISCUSSION

Histologically, granuloma annulare is characterized as a histio-monocytic granuloma associated with areas of necrobiosis (Civatte, 1967). Rheumatoid nodule has a similar histological appearance, with deep localization in the dermis and subcutaneous tissue and more pronounced, confluent necrobiosis with fibrinoid change. A previous review of granuloma annulare (Umbert & Winkelmann, unpublished

TABLE 3. Results of direct immunofluorescence in cutaneous sarcoidosis, necrobiosis lipoidica, and normal controls

Diagnosis	Case	Dermal–epidermal junction	Cytoid-like bodies	Superficial vessels	Dermis
Cutaneous sarcoidosis	1	IgM, fine granular	Neg.	Neg.	Fibrin
Necrobiosis lipoidica	2	Neg.	Fibrin	Fibrin	Neg.
	3	Neg.	Neg.	Neg.	Neg.
	4	C3, fibrin, fine granular	Neg.	Fibrin, C3	Fibrin
Normal controls (10 cases)	—	Neg.	Neg.	Neg.	Neg.

data) revealed well-developed granulomatous and pallisading structures or even an 'allergic' granuloma. This spectrum of granuloma is well documented as being characteristic of several human hypersensitivity diseases. In granuloma annulare, perivascular cuffing of mononuclear infiltrate is often found (Umbert & Winkelmann, unpublished data), thus pointing to the possibility of a cell-mediated immunity mechanism. In addition, we observed polymorphonuclear infiltrates only in areas that were already necrobiotic.

The major findings in previous histochemical studies of granuloma annulare have been corroborated by our recent histochemistry and electron microscopy studies (Umbert & Winkelmann, unpublished data), which demonstrated the presence of lymphoblast-like lymphocytes and activated macrophages associated with connective tissue changes in the dermis. The mononuclear phagocyte is a multipotential cell (Page, Davies & Allison, 1974), and its activation is the central event in a chronic inflammatory and immunopathological process such as granuloma annulare. Histochemical study demonstrates acid phosphatase and other lysosomal enzymes associated with the granuloma (Umbert & Winkelmann, unpublished data).

The cause of the stimulus (or stimuli) that activates the mononuclear cells is unknown. The mononuclear phagocyte has the capacity to respond to different stimuli: antigenic substances (Bloom & Bennett, 1966), endotoxin (Wilton, Rosenstreich & Oppenheim, 1975), double-strand RNA, or lymphocyte-derived factors (Poulter & Turk, 1975). Recently, collagenolytic activity has been demonstrated in homogenates of rheumatoid nodules, which are capable of clearing acid-soluble tropocollagen at neutral pH (Dabbous *et al.*, 1974). Activated macrophages secrete collagenase (Wahl *et al.*, 1975; Werb & Gordon, 1975b), elastase (Werb & Gordon, 1975a), and plasminogen (Unkeless, Gordon & Reich, 1974) and generate other inflammatory mediators (Page *et al.*, 1974) such as Hageman factor, which is the initial step for the activation of complement, blood coagulation, and the fibrinolytic system (Schubert & Hamerman, 1968). The data suggest that a multi-enzyme system might be involved in the necrobiosis of nodules.

Direct IF techniques provide an easy way to detect antigen–antibody complexes and complement in the tissues. Our studies of granuloma annulare do not show immune complex disease or significant vasculitis as in the reactions associated with immediate hypersensitivity. The rare presence of immunoglobulins in the dermis or vessels does not correlate with the special pathological changes or course of granuloma annulare. The principal finding of extensive fibrin deposition is consistent with recent studies demonstrating that fibrin accumulation is a prominent and consistent feature of delayed hypersensitivity skin reaction, as in the Kveim reaction and allergic contact dermatitis in man (Salo

& Hannuksela, 1972; Colvin *et al.*, 1973). This pattern differed sharply from the intraluminal thrombi and vessel wall deposits of fibrin described in antibody-mediated reactions. Furthermore, direct evidence exists for the participation of the clotting system in cell-mediated hypersensitivity and that anticoagulants inhibit delayed reactions in animals (Schwartz & Zimmerman, 1971).

The presence of extensive extravascular deposits of fibrin mass is a distinctive and consistent feature of delayed hypersensitivity skin reaction in man (Colvin & Dvorak, 1975) and in sarcoidosis (Salo & Hannuksela, 1972). The fibrin mass detected was not unpolymerized fibrinogen because of its fibrillar appearance and its characteristic periodicity as seen in electron microscopy (Dvorak *et al.*, 1974). Recently, studies (Colvin & Dvorak, 1975) with radioactive tracers and with IF were employed to detect and quantify fibrinogen–fibrin deposition and albumin deposition in cell-mediated immunity in the guinea-pig. Classical delayed hypersensitivity reactions to old tuberculin and to the azobenzene arsonate hapten were characterized by a progressive increase in the fibrinogen and paralleled the development of induration and erythema. The retention and resulting accumulation of fibrinogen in the delayed skin reaction seem related to a local activation of the clotting system, with polymerization of fibrinogen into insoluble fibrin. In addition to increased formation of fibrin, inhibition of fibrinolytic pathways also might have a role, as suggested by the reduction in the vessel-associated plasminogen activator in other inflammatory processes (Kwaan, 1966). The simplest explanation is that extravascular fibrin formation is a necessary consequence of increased vascular permeability, with extravasation of fibrinogen and other clotting factors. Hageman factor would be expected to be activated on contact with collagen (Wilner, Nossel & LeRoy, 1968) or perhaps with other connective tissue components. In addition, clotting could be initiated by lymphokines (Maillard, Pick & Turk, 1972). We have found lymphokines in the sera of patients with granuloma annulare (Umbert, Belcher & Winkelmann, 1976).

We would like to explain our findings by suggesting the possibility that the cellular hypersensitivity mechanism has an important role in granuloma annulare. Most recent studies (David, 1975) have shown that *in vivo* activation of macrophages requires the interaction of specific T-lymphocytes with appropriate antigen, and once activated, the macrophages may exhibit some nonspecific activity. Lymphocytes participating in cellular hypersensitivity produce a wide range of soluble mediators, some of which may interact with macrophages, causing the release of factors that induce tissue damage and destruction. The recruitment of mononuclear phagocytes into the sites of the delayed hypersensitivity reaction depends, in part at least, on chemotactic factors released by sensitized lymphocytes. These cells also produce and liberate the migration inhibition factor, which helps retain the mononuclear phagocytes at the site of inflammation and stimulates their activity. Thus, products of sensitized lymphocytes can induce macrophages to release enzymes, which may cause tissue damage and degradation together with fibrin formation at sites of chronic inflammation, such as granuloma annulare, and release lymphokines, such as migration inhibition factor, into the circulation.

ACKNOWLEDGMENT

The immunofluorescent studies were done in the Cutaneous Immunopathology Laboratory, Department of Dermatology, Mayo Clinic, Rochester, Minnesota.

REFERENCES

BEER, W.E. & WILSON JONES, E. (1966) Granuloma annulare following tuberculin Heaf tests. *Transactions of the St John's Hospital Dermatological Society*, **52**, 68.

BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in Pemphigus and Bullous Pemphigoid*, p. 94. Charles C.Thomas, Springfield, Illinois.

BLOOM, B.R. & BENNETT, B. (1966) Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science*, **153**, 80.

CIVATTE, J. (1967) *Histopathologie Cutanée*. Flammarion, Paris.

COLVIN, R.B. & DVORAK, H.F. (1975) Role of the clotting system in cell-mediated hypersensitivity. II. Kinetics of fibrinogen/fibrin accumulation and vascular permeability changes in tuberculin and cutaneous basophil hypersensitivity reactions. *Journal of Immunology*, **114**, 377.

COLVIN, R.B., JOHNSON, R.A., MIHM, M.C., JR. & DVORAK, H.F. (1973) Role of the clotting system in cell-mediated hypersensitivity. I. Fibrin deposition in delayed skin reactions in man. *Journal of Experimental Medicine*, **138**, 686.

DABBOUS, M.KH., YAMANISHI, Y., MAEYENS, E., HASHIMOTO, K. & HARDISON, H. (1974) Collagenolytic activity in rheumatoid nodules. I. Effect on acid-soluble tropocollagen. *Acta dermato-venereologica*, **54**, 265.

DAVID, J. (1975) The phagocytic cell in host resistance. In: *A Monograph of the National Institutes of Child Health and Human Development* (Ed. by J.A. Bellanti and D.H.Dayton), p. 143. Raven Press, New York.

DICKEN, C.H., CARRINGTON, S.G. & WINKELMANN, R.K. (1969) Generalized granuloma annulare. *Archives of Dermatology*, **99**, 556.

DVORAK, H.F., MIHM, M.C., JR., DVORAK, A.M., JOHNSON, R.A., MANSEAU, E.J., MORGAN, E. & COLVIN, R.B. (1974) Morphology of delayed type hypersensitivity reactions in man. I. Quantitative description of the inflammatory response. *Laboratory Investigation*, **31**, 111.

HAIM, S., FRIEDMAN-BIRNBAUM, R., HAIM, N., SHAFRIR, A. & RAVINA, A. (1973) Carbohydrate tolerance in patients with granuloma annulare: study of fifty-two cases. *British Journal of Dermatology*, **88**, 447.

HAMMOND, R., DYESS, K. & CASTRO, A. (1972) Insulin production and glucose tolerance in patients with granuloma annulare. *British Journal of Dermatology*, **87**, 540.

JORDON, R.E., TRIFTSHAUSER, C.T. & SCHROETER, A.L. (1971) Direct immunofluorescent studies of pemphigus and bullous pemphigoid. *Archives of Dermatology*, **103**, 486.

KWAAN, H.C. (1966) Tissue fibrinolytic activity studied by a histochemical method. *Federation Proceedings: Federation of American Societies for Experimental Biology*, **25**, 52.

LEPPARD, B. & BLACK, M.M. (1972) Disseminated granuloma annulare: a variant in which the lesions involve the sun-exposed areas. *Transactions of the St John's Hospital Dermatological Society*, **58**, 186.

MAILLARD, J.L., PICK, E. & TURK, J.L. (1972) Interaction between 'sensitized lymphocytes' and antigen *in vitro*. V. Vascular permeability induced by skin-reactive factor. *International Archives of Allergy and Applied Immunology*, **42**, 50.

MOYER, D.G. (1964) Papular granuloma annulare. *Archives of Dermatology*, **89**, 41.

PAGE, R.C., DAVIES, P. & ALLISON, A.C. (1974) Participation of mononuclear phagocytes in chronic inflammatory diseases. *Journal of the Reticuloendothelial Society*, **15**, 413.

POULTER, L.W. & TURK, J.L. (1975) Studies on the effect of soluble lymphocyte products (lymphokines) on macrophage physiology. II. Cytochemical changes associated with activation. *Cellular Immunology*, **20**, 25.

SALO, O.P. & HANNUKSELA, M. (1972) Immunohistology of the Kveim reaction. *Annals of Clinical Research*, **4**, 169.

SCHUBERT, M. & HAMERMAN, D. (1968) *A Primer of Connective Tissue Biochemistry*. Lea & Febiger, Philadelphia.

SCHWARTZ, H.J. & ZIMMERMAN, T.S. (1971) The effect of ellagic acid on delayed hypersensitivity reactions in guinea pigs. *Journal of Immunology*, **106**, 450.

TOLMACH, J.A. (1961) Case 5: disseminated granuloma annulare (atypical). *Archives of Dermatology*, **84**, 167.

TRIFTSHAUSER, C., HAYDEN, D.W. & BEUTNER, E.H. (1970) Procedures for the immunization of goats with human immunoglobulins and complement. *International Archives of Allergy and Applied Immunology*, **38**, 315.

UMBERT, P., BELCHER, R.W. & WINKELMANN, R.K. (1976) Lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare. *British Journal of Dermatology*, **95**, 481.

UMBERT, P. & WINKELMANN, R.K. Histologic, histochemistry and ultrastructural studies of granuloma annulare (unpublished data).

UNKELESS, J.C., GORDON, S. & REICH, E. (1974) Secretion of plasminogen activator by stimulated macrophages. *Journal of Experimental Medicine*, **139**, 834.

WAHL, L.M., WAHL, S.M., MERGENHAGEN, S.E. & MARTIN, G.R. (1975) Collagenase production by lymphokine-activated macrophages. *Science*, **187**, 261.

WERB, Z. & GORDON, S. (1975a) Elastase secretion by stimulated macrophages. *Journal of Experimental Medicine*, **142**, 361.

WERB, Z. & GORDON, S. (1975b) Secretion of a specific collagenase by stimulated macrophages. *Journal of Experimental Medicine*, **142**, 346.

WILNER, G.D., NOSSEL, H.L. & LEROY, E.C. (1968) Activation of Hageman factor by collagen. *Journal of Clinical Investigation*, **47**, 2608.

WILTON, J.M., ROSENSTREICH, D.L. & OPPENHEIM, J.J. (1975) Activation of guinea-pig macrophages by bacterial lipopolysaccharide requires bone marrow-derived lymphocytes. *Journal of Immunology*, **114**, 388.

British Journal of Dermatology (1976) **95**, 493.

Hypersensitivity to bacteria in eczema

IV. CYTOTOXIC EFFECT OF ANTIBACTERIAL ANTIBODY ON SKIN CELLS ACQUIRING BACTERIAL ANTIGENS

W.E.PARISH,* ESTHER WELBOURN AND R.H.CHAMPION

Department of Dermatology, Addenbrooke's Hospital, Cambridge, and
The Lister Institute of Preventive Medicine, Elstree, Herts.

Accepted for publication 9 January 1976

SUMMARY

The sera of persons with generalized eczema (Whitfield-type) or with disseminated nummular eczema were examined for complement-activating antibacterial antibodies to test the hypothesis that some eczematous change results from an antibody-mediated cytotoxic reaction. Bacteria dying in the stratum corneum release soluble antigens, some of which diffuse into the stratum Malpighii and become firmly adsorbed to the epidermal cells. Antibacterial antibody and complement diffusing into the epidermis react with the antigens acquired by the cells and may induce vacuolation or lysis.

Phenol-extracted and freeze-press-extracted antigens (both containing teichoic acids) from *Staphylococcus aureus* and a micrococcus (Baird-Parker types S1 and M1 respectively) are adsorbed by monolayers of human skin, embryo or amnion. Cells acquiring the antigen(s) are severely damaged when treated with sera containing the appropriate antibacterial antibodies and complement. IgM complement-fixing antibody appears to be much more cytotoxic in this test than IgG. The cytotoxic activity of a serum is specific for the acquired bacterial antigen and appears to depend on a sufficient concentration of the effective antibody, and not on the presence of antibodies with special properties. Explants of full thickness skin treated with bacterial antigen extracts were unharmed by the antibodies that were cytotoxic for monolayers of skin cells treated with the same antigens.

The *in vitro* cytotoxic test should represent a potential *in vivo* cytotoxic phenomenon, because skin cell monolayers from two patients adsorbed bacterial antigen prepared from cultures obtained from the same patients, and were damaged by autologous serum containing anti-staphylococcal antibody and complement.

It seems probable that this may be an aggravating but not necessarily an initiating factor in many cases of eczema.

We previously reported that patients with generalized eczema (Whitfield-type) or with disseminated nummular eczema tended to have numerous staphylococci and micrococci on the skin surface, and

---

* Present address: Unilever Research, Colworth House, Sharnbrook, Bedford.

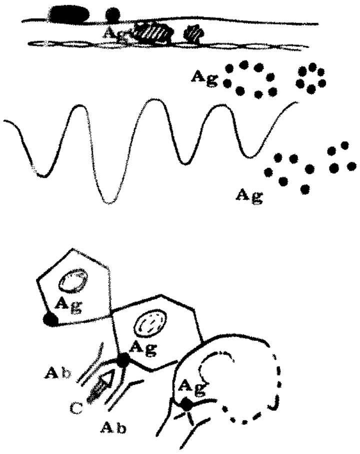


FIGURE 1. Diagrammatic representation of the cytotoxic response resulting from antibody and complement reacting with cell-acquired antigen. Soluble antigens from bacteria dying on the stratum corneum may penetrate the skin and become firmly adsorbed to the membranes of epidermal cells. Antibacterial antibody, with complement, reacting with the acquired bacterial antigen, induces epidermal cell damage.

sometimes had antigens of these bacteria bound to stratum Malpighii cells (Welbourn, Champion & Parish, 1976a). When examined by skin tests with the bacterial antigens, a high proportion of eczematous persons showed immediate, Arthus-like, or combined responses (Welbourn *et al.*, 1976a, b). The sera of many patients had complement-activating antibodies to staphylococci and micrococci (Parish, Welbourn & Champion, 1976).

The above findings were preparatory to the study reported here. We examined the possibility that some soluble antigens released from dead bacteria become firmly adsorbed to cells of the stratum Malpighii, and that antibody and complement diffusing through the dermo-epidermal junction of changed skin with eczema reacts with the acquired antigens, resulting in damage to or death of the epidermal cells, as depicted in Fig. 1.

Monolayers of human skin, embryo or amnion were treated with phenol-extracted or freeze-press extracted soluble antigens from a culture of staphylococcus or micrococcus. Both cultures were obtained from the skin of eczematous persons. The treatment for at least three hours was sufficiently long for antigens to bind to the cells if able to do so. After washing to remove unbound antigen, the cultures were treated with sera of patients containing antibody to the bacterial antigens and with complement.

MATERIALS AND METHODS

The patients, the preparation of bacterial antigen, and tests for bacterial antibodies have been described (Welbourn *et al.*, 1976a; Parish *et al.*, 1976). Tests were made with antigens from a culture of *Staphylococcus aureus* (Baird-Parker type S1) and of micrococcus (Baird-Parker type M1). Phenol extracts are presumed to be mainly teichoic acids because of the high content of phosphate (7·2%), precipitation with 1% DEAE-dextran, and spontaneous absorption to red cells (Parish *et al.*, 1976).

Proteins from both cultures were prepared from freeze-press extracts of packed, washed bacteria, by precipitation with cold 10% trichloracetic acid. The precipitate was washed once with 1% trichloracetic acid, dissolved in saline buffered pH 7·8 with Sorensen's buffer, and dialysed against this fluid. The protein was separated from carbohydrate on a Sephadex G150 column, the resulting eluate was dialysed against distilled water, and the solution together with material precipitated during dialysis was freeze-dried. The protein was reconstituted in Eagle's MEM medium at 500 μg/ml for treatment of cell cultures.

Cytotoxic tests on cell monolayers treated with bacterial antigens

Cell monolayers were prepared from trypsinized tissues in the normal manner. Human skin was surplus tissue removed during surgery. Samples were taken of normal skin from eczematous patients. Tissues were taken from the trunk and limbs of human embryos of 4–6 weeks gestation. Amnion was obtained from normal deliveries. Eagle's MEM medium with 20% normal human serum was used for the first 48–72 h, thereafter 10% normal serum.

Cells acquired bacterial antigens by washing the monolayer once with Eagle's medium, without serum, and treating them with 0·01 or 0·005% bacterial solution in Eagle's medium for 3 h at 37°C. The cells were either washed three times to remove uncombined antigens, or the medium was replaced with 0·01 or 0·005% bacterial substance in Eagle's medium plus 10% normal human serum for about 16 h (overnight) before washing off uncombined antigen.

The antigen treated cells were then tested with the patient's serum containing anti-bacterial antibody, either as 10% serum containing its native complement, or as 10% heated serum plus 10% fresh normal group AB serum containing complement. Changes in susceptible cells could be seen in 15–30 min, but all tests were incubated for 3 h. Control tests were always made of cells + antigen + normal serum and complement, cells without antigen + patient's serum, cells without antigen with change of normal medium. Fields of normal cells in the cultures (Rose chambers) were always marked before the test to eliminate bias when finding groups of abnormal cells present in all cultures.

Explants of skin and tests for skin auto-allergy. Fragments of full thickness skin were mounted in chicken plasma clots and cultured as described previously (Parish, Rook & Champion, 1965) except that Eagle's was used instead of Parker 199 medium. Sera of patients were tested for skin cytotoxic auto-antibodies as described before (Parish *et al.*, 1965).

Detection of bacterial antigen in treated cell monolayers. Cell monolayers treated with bacterial antigenic solutions were shown to have acquired bacterial antigens which were so firmly bound that washing the cells 12–20 times did not remove the antigens. Extracts of these antigen-treated, washed cells injected into rabbits induced formation of antibody to the acquired bacterial antigens.

RESULTS

Monolayers of human skin cells, and in a few instances human embryo or amnion cells, were treated with various antigenic extracts of staphylococci or micrococci, for 3 or 16 h, as described under Methods. The monolayers were then washed, and tested with the sera of eczematous patients which contained antibacterial antibodies, to determine whether or not the antibodies damaged cells that had irreversibly fixed or acquired the bacterial antigen.

Tests were made on monolayers treated with non-toxic protein antigens, phenol-extracted and freeze-press-extracted antigens. Subsequently full thickness skin explants were treated with phenol-extracted antigens to represent more closely events which may occur in skin *in vivo.*

Non-toxic protein antigens

Monolayers of human skin or of embryo kidneys which had been treated for 3 or 16 h with staphylococcal or micrococcal non-toxic proteins, up to 500 μg/ml concentration, and subsequently treated with serum from eczematous or normal persons with antibody to the relevant protein antigens, were unharmed. The cultured cells were shown to have bound the antigens sufficiently firmly that they were not eluted by washing, but the nature of the bond did not predispose the cells to damage by antibody and complement.

Phenol-extracted antigens (teichoic acids) and total antigen extracts

Monolayers of human skin cells treated with phenol-extracted antigens or with total, freeze-press, extracts, were severely damaged when subsequently tested with sera containing the relevant antibacterial antibodies and complement. Concentrations of 50 or 100 μg phenol extract/ml medium were consistently effective; 25 μg/ml was found to be sufficient antigen though not used in the cytotoxic tests. Greater concentrations of freeze-press extracts were required, 200–250 μg/ml. These concentrations of antigens were harmless to the cell cultures, even after incubation for 3 days. It made no difference to the results of the cytotoxic tests whether the cells were incubated with antigen for 3 or for 16 h before the treatment with antibody. Therefore the antigen was adsorbed fairly quickly.

Cytotoxic changes

Monolayers of normal skin cells usually were fibroblast-like, and in areas containing numerous cells they were elongated and formed wefts. In areas containing fewer cells, some were stellate and linked by numerous cytoplasmic processes, whereas others were hexagonal with fewer cytoplasmic processes. The differences in cell form probably reflect cell density and the age of the culture, rather than differences in origin from fibroblasts, macrophages or epidermal cells.

Observation under phase-contrast illumination of previously marked areas of normal cells showed that changing the medium for normal fresh medium or the addition of the patient's serum, or with the addition of the bacterial antigen solution, or the addition of bacterial antigen followed after 3 h by complement, induced no change or only slight changes in the cells. The slight changes were increased numbers of minute perinuclear vacuoles, or slight retraction of free cytoplasmic processes, i.e. processes not linked to those of other cells. Bacterial antigens occasionally induced larger perinuclear vacuoles, especially after 24 h. Changing the medium, even when at the correct temperature, was sufficient to cause such changes which were however sometimes so slight as to require close observation or a photographic record to detect them. Similar changes occurring with the test serum or antigen were therefore considered insignificant. The sera of a few patients caused more severe changes, and were not used in the investigation.

Cells acquiring bacterial antigen and treated with medium containing complement and patient's serum with antibacterial antibodies, showed changes in 15–30 min when examined by phase contrast illumination (Figs 2–5). There was a foam-like perinuclear vacuolation, dispersal of the cytoplasmic

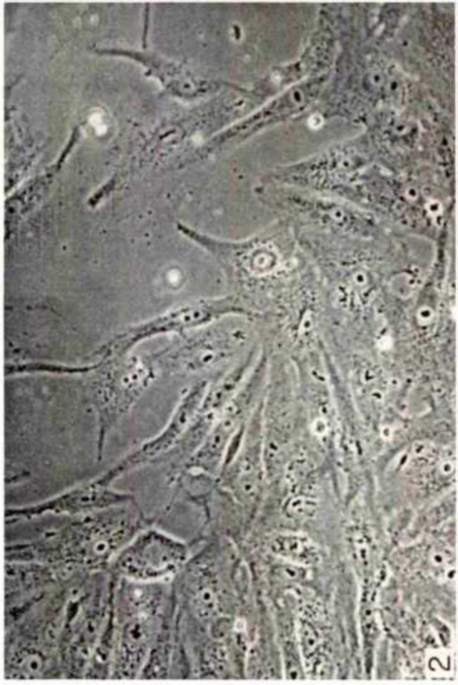
---

FIGURE 2. Monolayer of human skin cells, treated with phenol extracted staphylococcal antigen, and maintained in medium containing 10% normal human serum with very little anti-staphylococcal antibody. Cells have normal morphology. Phase-contrast.

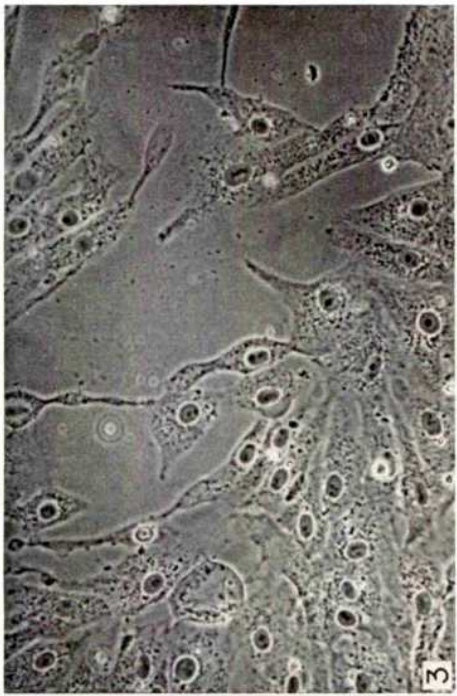
FIGURE 3. Monolayer of human skin cells. Same field as Fig. 8. 15 min after exchanging medium for 10% autologous fresh serum from skin donor containing complement-activating anti-staphylococcal antibody cytotoxic changes in cytoplasm and nuclei. Phase contrast.

FIGURE 4. Monolayer of human skin cells. Same field as Figs 8–9. 30 min after addition of autologous serum containing anti-staphylococcal antibody and complement. Blebs of cytoplasmic material exude from swollen cells. Phase contrast.

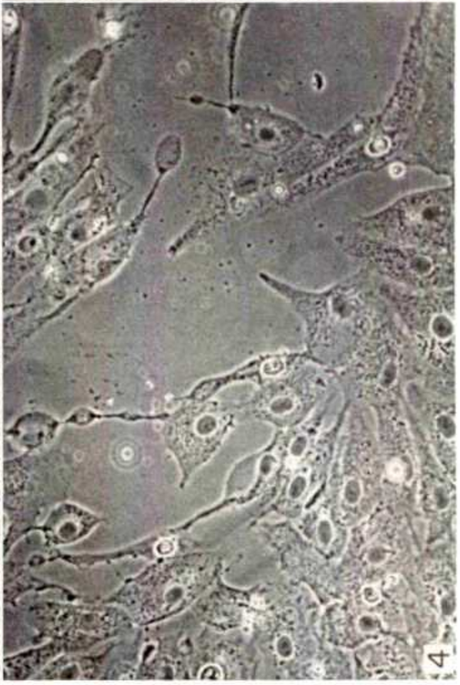
FIGURE 5. Monolayer of human skin cells. Same fields as Figs 8–10. 60 min after addition of autologous serum containing anti-staphylococcal antibody and complement. Cells severely damaged or killed. Phase contrast.



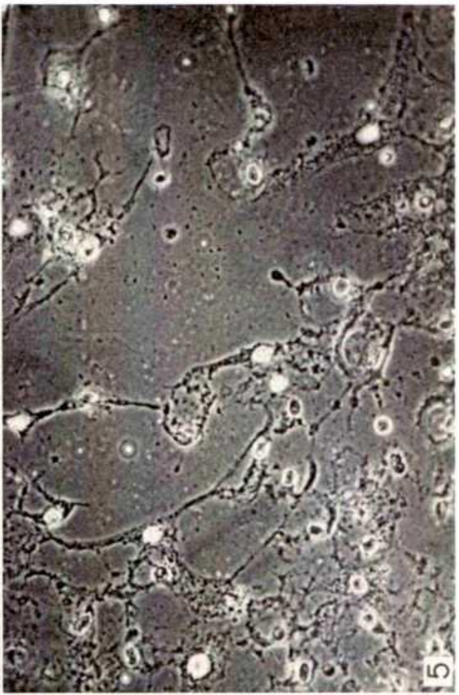
2



3



4



5

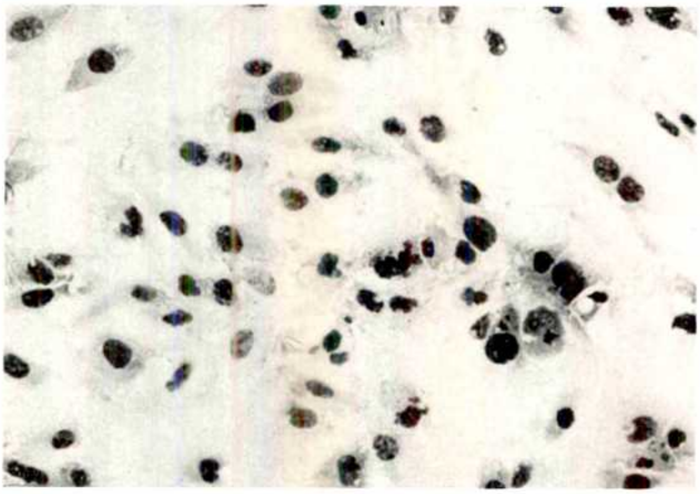


FIGURE 6. Human skin monolayer. No test treatment. Examples of some spontaneous changes observed in cultures; pyknosis and occasionally karyorrhexis of nuclei, and vacuolation of cytoplasm. Fixed, 80% alc. stained May-Grunwald Giemsa (× 380).

organelles towards the cell membrane and round the nucleus, thickening and retraction of the intercellular processes and occasional blebs. In some cells there were no further changes, but in most of the cultures the intercellular processes retracted further and became thinner leaving only a filament, or were shed; more blebs exuded from the cell, sometimes containing one or two organelles which vibrated within them, and the nuclear detail was obscured. Cells with more than one bleb or with a large bleb tended to rupture and release their contents, which floated in the medium as a raft of a nucleus surrounded by an irregular mass of granular cytoplasmic organelles. Cells which extruded less cytoplasm persisted, but were rounded and vacuolated, and incapable of mitosis if the culture was maintained. Most of each culture was severely damaged but some of the older cells in the culture survived and were capable of division after a lag period of 24–36 h.

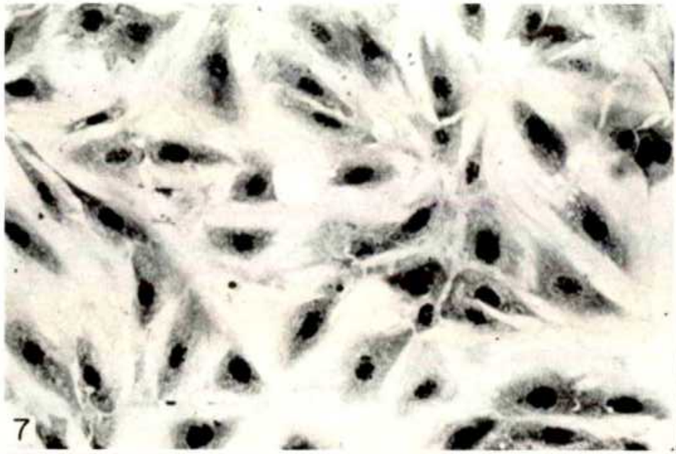
Similar changes were observed when the cultures were air-dried, fixed and stained after the test (Figs 6–10). After treatment with antigen and the appropriate antibody, many cells contracted, lost their definite outline, had pyknotic nuclei, and pale amorphous cytoplasm. A few cells appeared to escape damage and these showed mitoses if the culture was maintained. Fresh complement was essential for this severe cytotoxic damage.

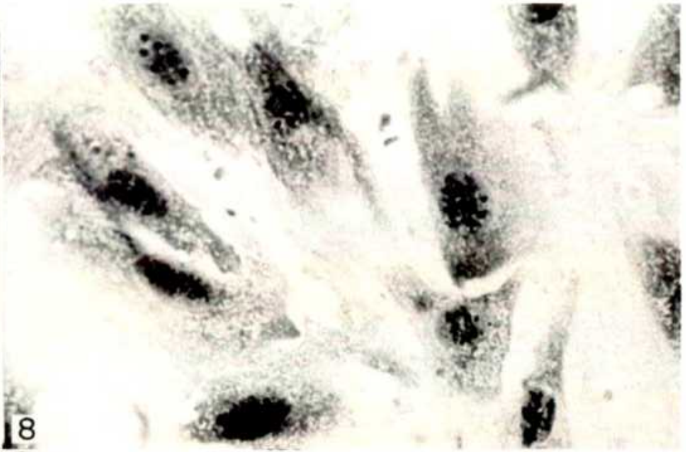
FIGURE 7. Human skin monolayer, treated with 50 μg staphylococcal phenol extract for 3 h and maintained 24 h in medium containing normal human serum, without antibody to staphylococcus. No harmful change and fewer spontaneous changes than depicted in Fig. 6. Fixed, 80% alc. stained May-Grunwald Giemsa (× 380).

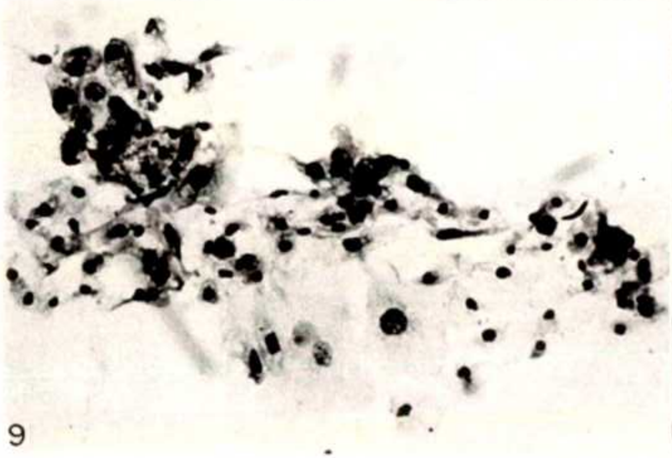
FIGURE 8. Human skin monolayer. Greater magnification of cells acquiring staphylococcal antigen, as in Fig. 7 (× 950).

FIGURE 9. Human skin monolayer. Culture treated with staphylococcal antigen as in Fig. 2. Then, instead of normal serum, treated with medium containing human serum with complement-activating anti-staphylococcal antibody. Most cells severely damaged or killed. Fixed 80% alc. stained May-Grunwald Giemsa (× 380).

FIGURE 10. Human skin monolayer. Greater magnification of cytotoxic change, as in Fig. 9 (× 950).


7


8


9

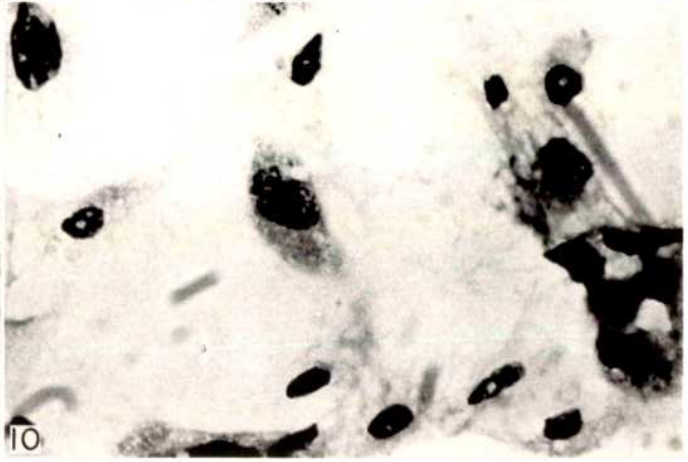

10

TABLE 1. Skin tests, antibacterial antibody titres and ability of patient's serum to damage monolayer cultures of human cells acquiring the relevant bacterial antigen *in vitro*

				Antibody titres					Cytotoxic test		
				PE				TE TCT	Test cells	Acquired antigen	Result
Patient	Test bacterium	Skin test Immediate	Skin test Arthus-like	Aggl.	γG	γM	C-lysis				
AM	Staph.	±	±	16	Not done		4	8	Homologous skin	TE	–
AS	Microc.	+	±	16	—	16	4	8	Homologous skin	TE	±
AA (Control)	Microc.	±	–	4	—	4	0	2	Homologous skin	TE	–
BJ	Staph.	±	±	16	64	16	8	32	Homologous skin	TE	±
									Homologous skin	PE	±
BI	Staph.	–	–	128	Not done		0	32	Homologous skin	TE	–
									Homologous skin	PE	–
BP	Staph.	±	–	32	Not done		8	128	Homologous skin	TE	–
BW	Microc.	±	–	8	16	32	0	8	Homologous skin	TE	–
CF	Microc.	±	–	16	—	16	0	0	Homologous skin	TE	–
HB	Staph.	+	–	16	—	32	64	64	Autologous skin	TE	+
									Autologous skin	PE	+
									Homologous skin	TE	+
									Homologous skin	PE	+
JJ	Staph.	±	–	32	64	32	64	8	Homologous skin	TE	+
									Homologous skin	PE	
	Microc.	±	–	8	128	16	16	n.d.	Homologous skin	PE	+
LS 1965	Staph.	–	–	256	Not done		64	1000	Homologous skin	PE	+
									Embryo	PE	+

TABLE I. (*cont.*)

1967	Staph.	+	±	32	—	—	8	256	Homologous skin	TE	–
									Homologous skin	PE	–
	Microc.	+	±	16	—	—	4	n.d.	Homologous skin	TE	–
									Homologous skin	PE	–
LP	Staph.	+	±	64	64	64	8	512	Homologous skin	TE	–
									Homologous skin	PE	–
MP	Staph.	+	–	8	16	8	8	4	Autologous skin	TE	–
									Homologous skin	PE	–
PE	Staph.	+	–	2	32	—	0	8	Homologous skin	TE	–
PF	Staph.	+	–	0	—	16	2	n.d.	Embryo	TE	–
PG	Staph.	–	–	32	128	64	64	256	Embryo	TE	+
									Amnion	TE	+
RL											
1968	Staph.	±	–	8	32	32	0	16	Homologous skin	TE	–
1969		±	–	8	> 1000	64	64	> 1000	Homologous skin	TE	+
RD	Staph.	+	–	4	64	32	16	128	Homologous skin	TE	–
SH	Staph.	+	+	32	256	—	16	128	Homologous skin	TE	+
									Embryo	TE	+
									Homologous skin	PE	±
SE	Microc.	+	–	64	256	128	16	n.d.	Homologous skin	PE	+
UJ	Staph.	–	±	256	Not done		8	128	Homologous skin	TE	–
WJ	Staph.	±	–	8	128	16	16	256	Embryo	PE	+
	Microc.	±	–	32	256	–	4		Embryo	PE	–
WC	Staph.	+	+	4	—	32	32	64	Autologous skin	TE	+
									Homologous skin	TE	+

Abbreviations. Staph = *Staph. aureus*, Baird Parker—type S1. Microc = Microccocus, Baird Parker—type M1.

PE = phenol extracted antigens, and TE = total (freeze-press) extracted antigens of the appropriate bacteria. TCT = tanned red cell agglutination test.

Reactions in skin tests and cytotoxic tests, + positive, ± weak, – negative. The + or – in immediate reaction indicates presence of absence of a weal, and in Arthus or Arthus-like (4 hour) readings, induration and congestion.

Cytotoxic changes in homologous and autologous cells

The use of homologous skin truly represented the changes occurring when the donor's serum was tested against the same donor's skin cells treated with the appropriate antigen. The sera of HB and WC induced similar cytotoxic changes in antigen-treated autologous and homologous skin, and the serum of MP was harmless to skin from both sources (Table 1). Monolayer cultures of human embryo and of amnion adsorbing the antigens were also susceptible to antibody-induced cytotoxic damage.

The cytotoxic changes observed *in vitro* were considered to be a suitable model for *in vivo* changes, because in two patients, HB and WC (Table 1), the skin cells were treated with antigen obtained from staphylococci cultured from the same donors, and each antigen-treated culture was tested with autologous serum, which damaged the cells.

Specificity of the cytotoxic antibodies

The cytotoxic changes were induced by antibody specific for the acquired bacterial antigen, because cells not treated with the antigen were unharmed by the sera. Absorption of six sera with phenol-extracted antigen adsorbed onto red cells removed all, and acetone-treated bacterial particles removed much, of the agglutinin; both procedures removed the cytotoxicity for cells with acquired bacterial antigen, though fresh complement was added to the medium.

None of the patients (Table 1) had cytotoxic auto-antibody to skin when tested on full depth explants.

Properties of the sera inducing cytotoxic changes in cells acquiring antigen

All sera cytotoxic in this test had complement-lysis titres of 16 or greater for red cells adsorbing phenol-extracted antigen. The tanned cell agglutination titre was unrelated to the cytotoxic activity and the complement-lysis test titre. One serum (JJ) with a tanned cell agglutination titre of 8 to total antigen extract was cytotoxic, whereas others with titres of 128 (BP and UJ), 256 (LS) and 512 (LP) were harmless (Table 1).

Antiglobulin tests on 8 of the 9 sera with agglutinins to phenol-extracted antigen and which were cytotoxic, showed that five sera had both IgG and IgM agglutinins (JJ, PG, RL, SE and WJ) and one had IgG only (SH). The titres of the complement-lysis tests were fairly consistent with those of the IgM antiglobulin tests. Furthermore, two sera with IgM antibody only were cytotoxic, and the serum SH, with an IgG antibody titre of 256 without IgM agglutination, had a complement-lysis titre of only 16, which may have been mediated by IgM present in too low a concentration to be detected in the antiglobulin test at a titre greater than 32, which was the direct agglutination titre for that serum. The cytotoxic activity of the IgM antibody was further shown by separating three sera on Sephadex G 200 columns. The IgM fraction of serum JJ contained all the cytotoxic antibody activity for cells acquiring staphylococcal or micrococcal antigens, though the IgG antibody titres, 64 and 128, were greater than those of IgM. Similarly the IgM fraction of serum PG, at a titre of 64, had all the cytotoxic activity though the IgG antibody had a greater titre at 128. The cytotoxic activity however was not confined to IgM in all sera, because in serum RL (1969 sample) nearly all the cytotoxic activity was in the IgG antibody which had a titre to staphylococcal antigen >1000, and only a trace of cytotoxic activity in IgM which nevertheless had a fairly high titre of 64 as measured in these tests. It is noteworthy that the IgG antibody in serum RL appeared to be mainly a nonagglutinating form, which was very unusual in antibodies to teichoic acids and related antigens.

There is an indication that it is the amount rather than the quality of the antibody, particularly of IgM complement-activating antibodies, that determines the ability of a serum to damage cells acquiring the antigen. Sera with IgM agglutinating titres and complement-lysis titres of 8 or lower were ineffective, whereas those greater than 16 were usually cytotoxic (one exception was RL sample 1969). When serum samples were taken at a 2-year interval from LS, and a one-year interval from RL, in each patient the antibodies were harmful only when the titres were high, though an IgG antibody mediated the cytotoxic change of RL serum.

Relation of cytotoxic antibody and skin test

No relation was found between the immediate skin test result, or Arthus-like, or delayed skin test responses, and the ability of the donor's serum to induce cytotoxic change in cells acquiring the bacterial antigens (Table 1), just as no relation was found between skin test responses and antibacterial antibody titres (Parish *et al.*, 1976).

Phenol-extracted antigens adsorbed to skin explants

Samples of normal skin were examined by immunofluorescence to show that they contained antigens of staphylococci and micrococci, as described above. Fragments of the same skin samples were cultured as explants in medium with 10% patient's serum containing complement, or with the addition of another 10% normal fresh group AB serum. Though skin samples were found to have traces of the relevant bacterial antigens, the explants grew normally for the 7 days of examination.

Further skin explants were treated for 3 h with 100 μg/ml bacterial antigen in Eagle's tissue culture medium, and for a further 16 h with the same antigenic medium with the addition of 10% normal serum, and were washed ten times before fragments were cultured. Addition of the patient's serum to form 10% of the medium in the presence of complement, induced no cytotoxic changes for 7 days.

The sera of four persons with antibacterial antibodies, two of whom had antibody to antigens of staphylococcus and two with antibody to micrococci, were tested. All four sera induced cytotoxic changes in antigen-treated skin cell monolayers. They were all harmless to explants of whole skin, even when the explants were previously treated with bacterial antigens.

DISCUSSION

This study shows that human skin cells will firmly adsorb some bacterial antigens, including those of staphylococci and micrococci which frequently occur on skin, and that antibacterial antibody and complement reacting with the acquired antigens damage the skin cells (Fig. 1).

For this to occur the patient must have formed sufficient complement-activating antibody to the appropriate bacterial antigens. We previously showed that such antibodies were formed (Parish *et al.*, 1976) and that the patients showed hypersensitivity responses after skin tests with bacterial antigens (Welbourn *et al.*, 1976a, b). The antibody must diffuse through the dermo–epidermal 'barrier', which was shown to occur in eczematous skin by immunofluorescence (Welbourn *et al.*, 1976a), and react with bacterial antigen adsorbed to and persistent on the epidermal cells, as shown in this report to occur on skin cell monolayers *in vitro*.

The bacteria most frequently colonizing the skin are staphylococci, micrococci and diphtheroids (Marples, 1965, 1969; Somerville, 1969; Noble & Somerville, 1974). The 'unaffected' skin of eczematous persons is colonized by more *Staph. aureus* organisms than is the skin of normal persons (Hare & Cook, 1961; Noble & Davies, 1965; Müller, 1969; Noble, 1971; Wilson, White & Noble, 1971), and numerous bacteria, particularly *Staph. aureus*, occur on moist eczematous lesions (Pillsbury & Nichols, 1946; Storck, 1948). The prevalence of *Staph. aureus* and micrococci was observed on the affected and clinically unaffected (though physiologically changed) skin of the eczematous patients that were examined (Welbourn *et al.*, 1976a). The large numbers of organisms and the increased moisture of eczematous skin are likely to predispose to percutaneous absorption of bacterial antigens. Our immunofluorescence tests showed that absorption occurred (Welbourn *et al.*, 1976a).

Thus epidermal cells may adsorb bacterial antigens which predispose them to damage by antibody to the acquired antigen and complement, in a manner similar to red cells which adsorb certain bacterial substances and are then agglutinated by antibacterial antibody, or lysed by the antibody and complement. Red cells adsorb lipopolysaccharides of Gram negative organisms (Keogh, North & Warburton, 1948; Fisher & Keogh, 1950; Davies *et al.*, 1958), polysaccharides of *Mycobacterium tuberculosis* (Middlebrook, 1950; Hall & Manion, 1951; Meynell, 1952) and polysaccharides, or more probably teichoic acids, from streptococci (Moskowitz, 1966; Goldstein & Caravano, 1967; Parish & Rhodes 1967; Ne'eman & Ginsburgh, 1972a, b). The appropriate antibodies to these antigens agglutinate the red cells which adsorbed the antigens, and lyse them when treated with complement. Nucleated tissue cells also adsorb some bacterial antigens and thereby become susceptible to damage by antibacterial antibodies and complement (Dishon *et al.*, 1967; Parish, 1972). One such substance is the teichoic acid extracted by phenol from streptococci. The cytotoxic complement-activating antibodies are mainly IgM (Ne'eman & Ginsburg, 1972a, b) as we found for the similar phenol-extracted antigen from staphylococci in this study.

Polysaccharides and teichoic acids persist in living tissues for several weeks (Kaplan, Coons & Deane, 1950; Wood & White, 1956; Cotran, 1963) whereas most proteins including streptococcal M protein are removed in 2–3 days. Macrophages *in vitro* can degrade Group A streptococcal M protein within 5 h, leaving diffuse M material within cell vacuoles (Gill & Cole, 1965), whereas the cell walls may persist in muscle and macrophages *in vivo* for 6–9 weeks (Adler *et al.*, 1973). Therefore epidermal cells adsorbing persistent antigen would remain susceptible to immunological cytotoxic damage, and the adsorption may be accumulative—thereby increasing the susceptibility.

Though the cytotoxic effect was observed regularly in tests on monolayers, it did not occur when explants of skin were treated with appropriate bacterial antigens and with sera that induced changes in monolayers. These explants were not damaged even though they were shown to have adsorbed the antigens. The cells of organized tissues, like epidermis, may be protected from such damage by being closely packed; alternatively there may be inhibitors of this type of change in tissues, which are destroyed in the preparation of monolayers.

Clinical significance of antibacterial antibodies in eczema

Bacterial antigens may prepare the epidermis for a cytotoxic reaction in all persons, whether normal or with skin disorders, who have sufficient numbers of bacteria colonizing the skin and who have formed the appropriate antibodies.

In practice this type of cytotoxic reaction is only likely to occur when the numbers of bacteria and diffusion of antibody into the epidermis are greatly exaggerated above normal, as in eczema. In the acute phase of generalized eczema the skin is moist, probably as a result of autolysis of degenerate cells and of increased vascular permeability. Hydration of the epidermis, particularly the corneal squames, provides optimal conditions for growth of bacteria, e.g. the staphylococci and micrococci abundantly present on some eczematous skin; this results in excessive amounts of bacterial antigen. The hydration of the skin will also enhance absorption of some of the soluble bacterial antigen into the epidermis. Concomitantly, increased vascular permeability and epidermal inflammatory change increase the diffusion of immunoglobulin, including antibacterial antibody, and complement into the epidermis. Epidermal cells which adsorb bacterial teichoic acids or polysaccharides onto their membranes will be killed or damaged when antibacterial antibody and complement react with the adsorbed antigen.

As the cytotoxic reaction depends upon the greatly increased growth of bacteria on the skin, and upon the increased vascular permeability predisposing to epidermal diffusion by antibody, the reaction is only likely to occur in skin that is already damaged. Therefore, the phenomenon probably potentiates eczematous change, and does not initiate it.

For this cytotoxic reaction to occur, the patient must have sufficient complement-activating antibacterial antibody. However, it is possible that a similar cytotoxic change may be induced by lymphocytes, specifically activated by bacterial antigen, reacting with bacterial antigens adsorbed to epidermal cells. Such lymphocytes could initiate as well as potentiate the eczematous reaction in a typical delayed hypersensitivity response.

Whether the hypersensitivity to bacteria promoting epidermal cell damage in eczema is induced by antibody or by lymphocytes, its occurrence justifies the trial of antibiotics in treatment of disseminated eczema, and is an explanation of the undoubted success of antibiotic treatment in some cases of persistent eczema.

ACKNOWLEDGMENTS

We are grateful to the Smith Kline & French Foundation for research grants to E.W. and W.E.P., and also to the Addenbrooke's Hospital Research Fund for a further grant to E.W., without which help this and related long-term studies could not have been completed.

We are grateful for advice and support from Drs Arthur Rook and Granville R.E.Naylor, and also to Drs Arthur Rook and Stephen Roberts for referring patients for the investigation. We thank Dr M.H.Gleeson-White, Mr S.T.Haslam and the Bacteriological Laboratory for the identification of the bacteriological cultures, and the following staff of the Lister Institute for much careful technical assistance—Miss Marisa Walisiewick, Mrs Gillian Wardle, Mrs Geraldine Yates, Mr Kenneth Veal, Mr Michael Scott and Miss Susan Hedges. We thank the Junior Staff of the Dermatology Department, Addenbrooke's Hospital for their help, and we especially thank the patients and the controls for their cooperation for so many years. Finally, we thank Mrs Mary Bangart for careful help in the preparation of the manuscripts.

REFERENCES

ADLER, E., HELLER, H., WIENER, E., MASOVER, A., FRIEDMAN, S., LAHAV, M., NE'EMAN, N. & GINSBURG, I. (1973) Degradation of ^{14}C-labelled group A streptococci and micrococci in muscular lesions in the mouse. *Israel Journal of Medical Sciences*, **9**, 483.

COTRAN, R.S. (1963) Retrograde proteus pyelonephritis in rats. Localization of antigen and antibody in treated sterile pyelonephritic kidney. *Journal of Experimental Medicine*, **117**, 813.

DAVIES, D.A.L., CRUMPTON, M.J., MACPHERSON, I.A. & HUTCHISON, A.M. (1958) The adsorption of bacterial polysaccharides by erythrocytes. *Immunology*, **2**, 157.

DISHON, T., FINKEL, R., MARCUS, Z. & GINSBURG, I. (1967) Cell-sensitizing products of streptococci. *Immunology*, **13**, 555.

FISHER, S. & KEOGH, E.V. (1950) Lysis by complement of erythrocytes which have adsorbed a bacterial component and its antibody. *Nature (Lond.)*, **165**, 248.

GILL, F.A. & COLE, R.M. (1965) The fate of a bacterial antigen streptococcal M protein (and the streptococcal M protein) after phagocytosis by macrophages. *Journal of Immunology*, **94**, 898.

GOLDSTEIN, I. & CARAVANO, R. (1967) Determination of anti Group A streptococcal polysaccharide antibodies in human sera by an hemagglutination technique. *Proceedings of the Society for Experimental Biology and Medicine*, **124**, 1209.

HALL, W.H. & MANION, R.E. (1951) Hemagglutinins and hemolysins for erythrocytes sensitized with tuberculin in pulmonary tuberculosis. *Journal of Clinical Investigation*, **30**, 1542.

HARE, R. & COOKE, E.M. (1961) Self-contamination of patients with staphylococcal infections. *British Medical Journal*, **ii**, 333.

KAPLAN, M.H., COONS, A.H. & DEANE, H.W. (1950) Localization of antigen in tissue cells. III. Cellular distribution of pneumococcal polysaccharides types II and III in the mouse. *Journal of Experimental Medicine*, **91**, 15.

KEOGH, E.V., NORTH, E.A. & WARBURTON, M.F. (1948) Adsorption of bacterial polysaccharide to erythrocytes. *Nature (Lond.)*, **161**, 687.

MARPLES, M.J. (1965) *The ecology of human skin.* Springfield, Illinois. Thomas.

MARPLES, M.J. (1969) The normal flora of the human skin. *British Journal of Dermatology*, **81,** Suppl. **1,** 2.

MEYNELL, G.G. (1952) A sensitive modification of the Middlebrook–Dubos haemagglutination test. *Journal of Pathology and Bacteriology*, **64,** 647.

MIDDLEBROOK, G. (1950) A hemolytic modification of the hemagglutination test for antibodies against tubercle bacillus antigens. *Journal of Clinical Investigation,* **29,** 1480.

MOSKOWITZ, M. (1966) Separation and properties of a red cell sensitizing substance from streptococci. *Journal of Bacteriology*, **91,** 2200.

MÜLLER, E. (1969) Ecology of *Staphylococcus aureus* on the human skin surface. V. *Staphylococcus aureus* artificially inoculated upon the skin surface after extraction with ether, of patients with dermatoses and during systemic administration of antibiotics. *Archiv für klinische und experimentelle Dermatologie*, **233,** 376.

NE'EMAN, N. & GINSBURG, I. (1972a) Red cell-sensitizing antigen of Group A streptococci. I. Biological and chemical properties. *Israel Journal of Medical Sciences*, **8,** 1799.

NE'EMAN, N. & GINSBURG, I. (1972b) Red cell-sensitizing antigen of Group A streptococci. II. Immunological and immunopathological properties. *Israel Journal of Medical Sciences*, **8,** 1807.

NOBLE, W.C. (1971) The contribution of individual patients to the spread of infection. *British Journal of Dermatology*, **85,** 24.

NOBLE, W.C. & DAVIES, R.R. (1965) Studies on the dispersal of staphylococci. *Journal of Clinical Pathology*, **18,** 16.

NOBLE, W.C. & SOMERVILLE, D.A. (1974) *Microbiology of human skin.* W.B. Saunders, London.

PARISH, W.E. (1972) Host damage resulting from hypersensitivity to bacteria. In: 'Microbial Pathogenicity in man and animals'. *Symposium of the Society for General Microbiology*, **22,** 157.

PARISH, W.E. & RHODES, E.L. (1967) Bacterial antigens and aggregated gamma globulin in the lesions of nodular vasculitis. *British Journal of Dermatology*, **79,** 131.

PARISH, W.E., ROOK, A.J. & CHAMPION, R.H. (1965) A study of autoallergy in generalized eczema. *British Journal of Dermatology*, **77,** 479.

PARISH, W.E., WELBOURN, E. & CHAMPION, R.H. (1976) Hypersensitivity to bacteria in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci. *British Journal of Dermatology* **95,** 285.

PILLSBURY, D.M. & NICHOLS, A.C. (1946) Bacterial flora of the normal and infected skin: an evaluation of various methods of performing skin cultures. *Journal of Investigative Dermatology*, **7,** 365.

STORCK, H. (1948) Experimentelle Untersuchungen zur Frage der Bedentung von mikroben in der Ekzemgenese. *Dermatologica (Basel)*, **96,** 177.

SOMERVILLE, D.A. (1969) The effect of age on the normal bacterial flora of the skin. *British Journal of Dermatology* **81,** Suppl. **1,** 14.

WELBOURN, E., CHAMPION, R.H. & PARISH, W.E. (1976a) Hypersensitivity to bacteria in eczema. I. Bacterial culture, skin tests, and immunofluorescent detection of immunoglobulins and bacterial antigens. *British Journal of Dermatology*, **94,** 619.

WELBOURN, E., CHAMPION, R.H. & PARISH, W.E. (1976b) Hypersensitivity to bacteria in eczema. III. Arthus-like responses to bacterial antigens without specific antibody. *British Journal of Dermatology*, **95,** 379.

WILSON, P.E., WHITE, P.M. & NOBLE, W.C. (1971) Infections in a hospital for patients with diseases of the skin. *Journal of Hygiene (Cambridge)*, **69,** 125.

WOOD, C. & WHITE, R.G. (1956) Experimental glomerulo-nephritis produced in mice by subcutaneous injections of heat-killed Proteus mirabilis. *British Journal of Experimental Pathology*, **37,** 49.

British Journal of Dermatology (1976) **95**, 507.

The ultrastructure of the human epidermis following ultracentrifugation

G.H.FINDLAY AND N.V.D.W.LIEBENBERG*

Department of Dermatology, University of Pretoria, and * Council for Scientific and Industrial Research, Pretoria, South Africa

Accepted for publication 29 March 1976

SUMMARY

Ultracentrifugation of the human epidermis was undertaken to uncover the forces responsible for the establishment and maintenance of the supranuclear melanin cap in basal cells. The wide spacing and elasticity of the tonofibrils caused a selective trapping of melanosomes in this region. At the same time the experiments produced pictures resembling dedifferentiation of the plasma membrane as in the fetal state, and differentiation of the tonofibrillar mass resembling the granular and horny layers. Once the tonofibrils had retracted and massed together, the order of intracellular sedimentation was: melanosome, chromatin, mitochondrion, tonofibril and ribosome.

Our study was aimed at finding out how melanosomes came to be aggregated in the form of a supranuclear cap in the epidermal basal cell. As an intracellular sunscreen, these pigment particles shield the nucleus from ultraviolet damage, yet there is little known about the way pigment occupies and maintains this functional position. To clarify the question, we attempted a forcible displacement of the melanin cap by ultracentrifugation in fresh human epidermal tissue.

During these experiments a mixed picture resembling differentiation and dedifferentiation was induced in the epidermal cells, yielding insights into cell strength under acceleration and compression as well as the interaction of cytoplasmic structures in epidermal cell architecture.

METHODS

Ultracentrifugation. Freshly excised sheets of normal adult Negro skin comprising the epidermis with part of the dermis were obtained from surgical donor sites during skin grafting operations. These specimens, placed with the external surface down or up on filters (paper, millipore), were centrifuged for 30 min at 6°C in saline or buffer followed by an identical recentrifugation in a fixative (e.g. 10% formalin), and compared with controls. By varying the forces from 700 to 273,000 *g*, an interesting range was found to lie between 100,000 and 150,000 *g*. In the final procedure under these conditions, two further variations were attempted using different rotors. With the Beckman L_2-65B ultracentrifuge, the Spinco SW 65 preparative rotor and the Ti-14 zonal rotor were compared. In the former the first fluid medium (in these tests 0·14 M sodium chloride in 0·05 M phosphate buffer at pH 7·2) was replaced by the second (5% glutaraldehyde in pH 7·0 phosphate buffer with and without 10% sucrose) during a brief complete stop of centrifugation, and in the latter the exchange was made during continued spinning, lowered briefly to 10,000 *g*. No preferred method emerged from these choices, except perhaps that the zonal rotor preserved the tissue better from disruption, there being less acceleration and deceleration with its use. No other variations in composition of the centrifuging medium were tried.

Microscopy. Optical and electron microscopy was performed on uncentrifuged controls and centrifuged specimens placed face-up and face-down. Standard methods of preparation were used: haematoxylin–eosin and melanin procedures for optical microscopy, and post-fixing in osmic acid and uranyl acetate and lead citrate for contrasting with electron microscopy. A Philips EM301 electron microscope was used. Six experimental sets comprised the final data.

FINDINGS

Optical microscopy

Epidermal changes after ultracentrifugation consisted of compression, disruption and stratification. Compression simulated epidermal atrophy with straightening of the dermo-epidermal junction. Disruption included an intracellular disorganization with ballooning and rupture of cells, and at highest speeds, cellular shrinkage and dissociation. These alterations, particularly the formation of vacuoles and vesicles, were conspicuous suprabasally. Stratification was noted as a movement of dense nuclear chromatin with the gravitational force. Finer intracytoplasmic movements could not be resolved with optical microscopy.

Electron microscopy

Melanosomes. Uncentrifuged basal cells revealed a reciprocal concentration of melanosomes and tonofibrils: where melanosomes were aggregated, tonofibrils were sparse, and vice versa. In the supranuclear region of the melanosome cap, tonofibril bundles were more widely separated (Fig. 1).

During ultracentrifugation the supranuclear tonofibrillar mass with melanosomes trapped within it could evidently slip *en bloc* round the nucleus to a basal position, while the nucleus moved the opposite way, like the bubble in a spirit level.

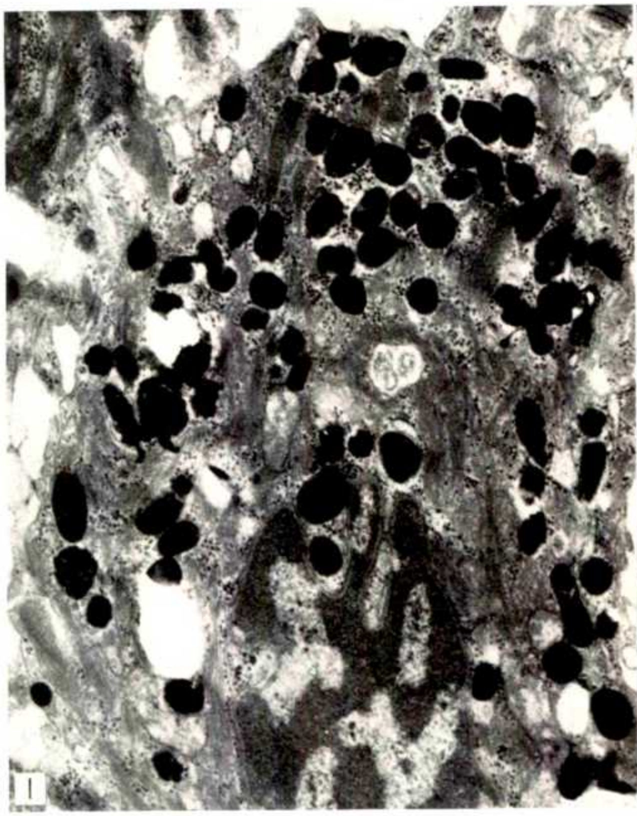
As the tonofibril system started to disintegrate, the melanosomes were released from its meshes so as to migrate independently in the gravitational field (Fig. 2). They were evidently the densest cell component, being seen to pile up, like a bank of hailstones, against the cell membrane or the nuclear membrane, driving pockets and dents at times into the latter.

Where free melanosomes lay (Fig. 3) spun-down and packed, with the melanosome membranes torn off, bloated and mutually interconnected, along with endoplasmic reticulum, vacuoles and lysosomes, they were flecked with erosions and cavities (vesicular bodies) where their electron density was lost.

Tonofibrils. Ultracentrifugation produced changes from slight disruption to complete sequestration of the tonofibrillar mass (Figs 4 and 5).

The changes, in order of their intensity, were: withdrawal of tonofibrils from desmosomes with desmosome loss, an agglutination of the fibril mass, decay of tonofibrils into a pale granular debris in the supranuclear zone releasing the melanosomes and revealing in these regions some possible lysosomal fragments and shreds of endoplasmic reticulum. Eventually the whole tonofibrillar mass was converted into a large 'bolus' of increasing density. This altered tonofibrillar mass sedimented in a position between the denser nucleus and less dense ribosomal mass.

In one experiment this mass showed marginal forked and branched 'stratum granulosum-like' opacifications in a Malpighian cell. In the angles of these branches some organelle relics were seen which might have been keratinosomes.



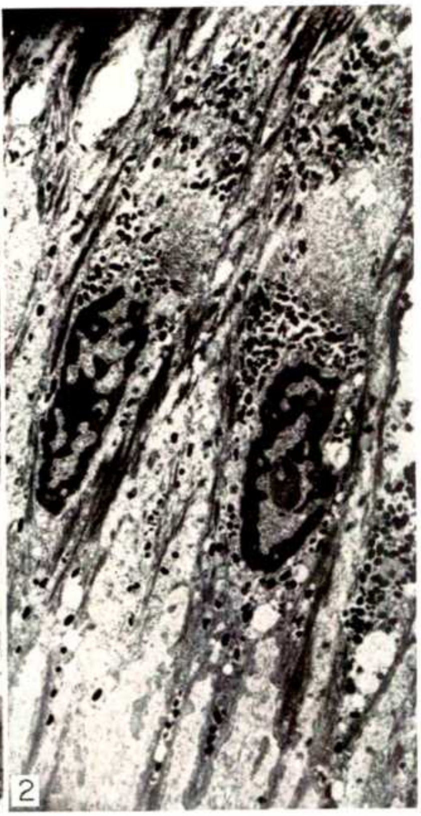


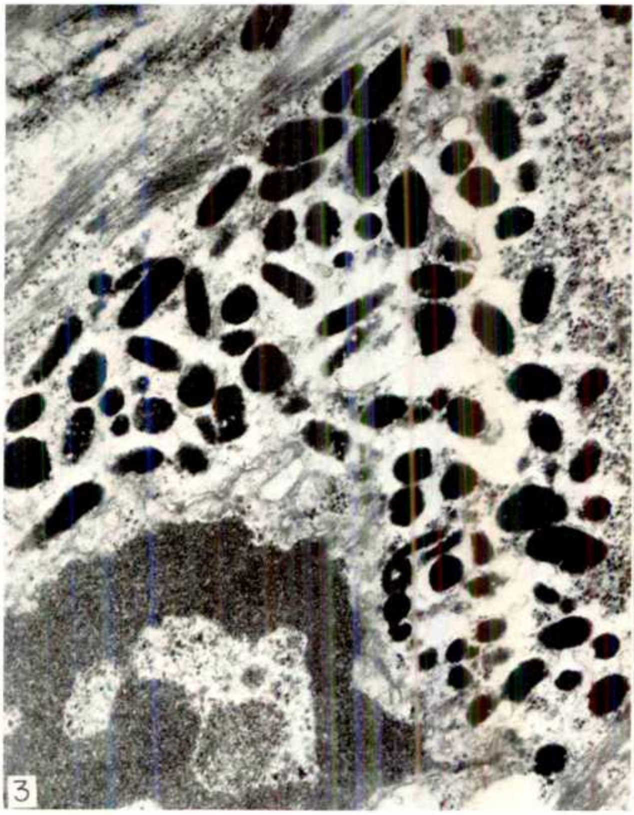
FIGURE 1. The normal supranuclear melanin cap in an epidermal basal cell (Negro). Melanosomes occupy much of the available space between tonofibril bundles in the outer part of the cell (× 27,000).

FIGURE 2. Supranuclear breakdown of the tonofibril complexes after downward ultracentrifugation. Melanosomes are released in this area, but not in the apex of the cells. Loose melanosomes lie piled above the nucleus with a layer of ribosomes on top of them (× 4000).

Plasma membrane. After ultracentrifugation the plasma membrane of the epidermal basal cells showed an extensive folding of the interdesmosomal regions to form a tightly interlocking villous mass. The cytoplasm in these villi was relatively clear, and formed an elaborate meshwork extending between the rather sparse residual desmosomes, and tightly engulfing the dendritic processes of Langerhans cells and melanocytes.

Endoplasmic reticulum. After centrifugation, loose intracellular villous structures, membrane shreds and pockets, balloonings and slit-like spaces were seen around the nucleus, and at sites of tonofibril disappearance or melanosome release.

Nucleus. There was no evidence of nuclear breakdown. The condensed chromatin blocks became detached from the nuclear membrane and tended to move in a unit along the gravitational field. The nucleus and endoplasmic reticulum, respectively, appeared to form a ball and socket type of arrangement which facilitated mechanical displacements of the nucleus during centrifugation, as would no doubt also occur during more natural types of temporary deformation.



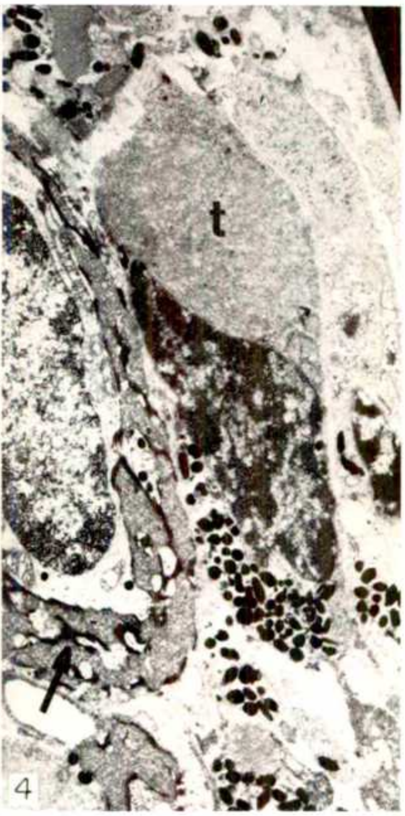


FIGURE 3. Detail of Fig. 2. Membranes round individual melanosomes are partly loosened, bloated and fused. Ribosomes remain largely outside this zone. Melanosomes are polymorphic and cavitated; condensed chromatin is retracted from the nuclear membrane. Tonofibrils are thinned out and have lost definition (× 27,000).

FIGURE 4. Granular transformation of the tonofibril mass (t) with condensations (arrow) suggesting dense portions as seen in the stratum granulosum (× 9000).

Other structures. Ribosomes, like melanosomes, were readily trapped in the tonofibrillary mesh. Many were set free as the tonofibrils condensed together, and were spun down as the slowest moving of the recognizable cell components. Mitochondria became evident when segregating out of the tonofibrillar mass. Their characteristics upon centrifugation were uncertain, but they appeared to be slightly less dense than the nucleus. After centrifugation a large number of trapped and bloated cavities, possibly lysosomal, were noted. The basal lamina region, hemidesmosomes and the dermis were not disrupted.

CONCLUSIONS

According to our observations, the supranuclear cap of melanosomes owes its position to a looseness in this area of the tonofibril meshwork in the basal keratinocyte. The looseness of the meshwork is confirmed by the greater ease of suprabasal cleft formation. The cap is evidently maintained there during stress because distortions of the keratinocytes spreading through the tonofibrillar system

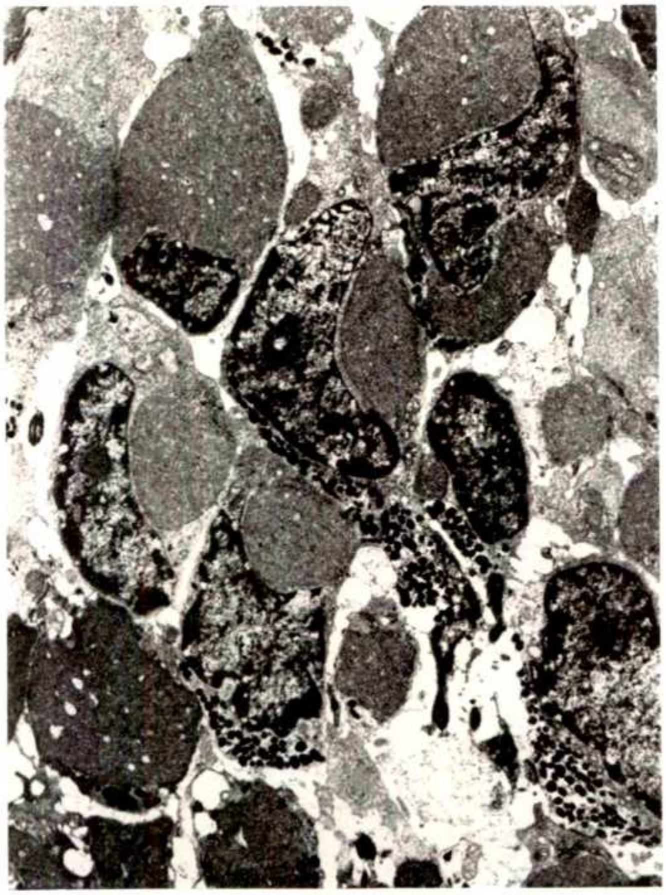


FIGURE 5. Dedifferentiation of the plasma membrane with loss of desmosomes. Tonofibrils are rolled up into an inclusion-like mass; melanosomes are released and are spun down to the base of the cells (× 6,000).

would tend to hold these particles trapped in the same position. The stressed tonofibrils, as such, give way first at the desmosomes, then later in the body of the cell. The tonofibril mass falls together in a granular mass, with the precipitation of stratum granulosum-like material. Injury to organelles evidently accelerates this pseudo-maturation in the epidermal cells. In the plasma membrane, with its villous change and desmosome retrogression, the change simulates dedifferentiation back to a picture seen particularly in the fetus. On the melanosomes, a normal destruction is evidently brought nearer at the same time as the tonofibrils are degraded. As long as the tonofibril system remains intact, the cell components maintain normal relationships during ultracentrifugation, but as the fibrils undergo this artificial maturation, the cell components are free to sediment out. The melanosome membranes bloat out and vesicular cavities appear in the melanosomes.

DISCUSSION

Physical forces exerted on the epidermis preceding study by electron microscopy have before now comprised high osmotic pressures, suction and friction *in vivo*. An epidermal intracellular canalicular system was unfolded (Hönigsmann & Wolff, 1973) by injecting solutions of high osmolarities into the dermis. They considered that endoplasmic reticulum was the structure forming these canals and vacuoles despite its being poorly visible in normal skin.

Suction stress (Hunter, McVittie & Comaish, 1974a) caused early herniation of membrane-bound vacuoles from the paranuclear region into the perinuclear space. Forced fluid uptake by keratinocytes suggested the existence of openings in the plasma membrane leading into the endoplasmic reticulum which dilated to form vacuoles.

Friction (Hunter *et al.*, 1974b) produced an initial retraction of tonofibrils from the cell periphery in the outer Malpighian keratinocytes with breakdown of the plasma membrane and desmosomes.

Ultracentrifugation in our study produced shrinkage, compression and an ultimate rearrangement of intracellular components. Comparing all the findings it appears that fully developed tonofibrils in the epidermal basal cell leave only three regions available for accumulation of fluid or particles—the perinuclear, supranuclear and peripheral regions. The *perinuclear* region provides the initial space for fluid increase. It also houses artificially introduced latex beads or foreign melanosomes (Wolff, 1973). The main direction for fluid expansion is into the tonofibril-free nuclear area. Until it ruptures, the endoplasmic reticulum provides the confining membranes. The *supranuclear* zone, with the Golgi apparatus, has a fibril pattern loose enough to contain many melanosomes, lysosomes and ribosomes. The *peripheral* region comprises the tops and sides only of columnar basal keratinocytes. Young, rapidly replicating basal keratinocytes in culture (Hashimoto & Kanzaki, 1974) show an entanglement of numerous interdigitating villi between the cells, and cell ripening runs parallel with desmosome increase and loss of villi. The final step in desmosome formation consists in the insertion of tonofilaments (Krawczyk & Wilgram, 1973) but when the cytoplasmic filaments are dense enough it may happen, as in the hair cortex, that the desmosomes dedifferentiate (Orwin, Thomson & Flower, 1973). Our findings show that these biological changes may be closely reproduced by compression of keratinocytes in the ultracentrifuge. If the desmosomal junctions could only have been maintained during centrifugation, the cytoplasmic changes might closely replicate the formation of a true granulosum in the basal and suprabasal keratinocyte.

ACKNOWLEDGMENTS

This work was done with the support of the Medical Research Council (South Africa). We are greatly indebted to Dr C.F. Albrecht for the ultracentrifugation of the specimens.

REFERENCES

HASHIMOTO, K. & KANZAKI, T. (1974) Surface structure of tissue cultured keratinocytes. *Journal of Ultrastructure Research*, **49**, 252.

HÖNIGSMANN, J. & WOLFF, K. (1973) Continuity of intercellular space and endoplasmic reticulum of keratinocytes. *Experimental Cell Research*, **80**, 191.

HUNTER, J.A.A., MCVITTIE, E. & COMAISH, J.S. (1974a) Light and electron microscopic studies of physical injury to the skin. 1. Suction. *British Journal of Dermatology*, **90**, 481.

HUNTER, J.A.A., MCVITTIE, E. & COMAISH, J.S. (1974b) Light and electron microscopic studies of physical injury to the skin. 2. Friction. *British Journal of Dermatology*, **90**, 491.

KRAWCZYK, W.S. & WILGRAM, G.F. (1973) Hemidesmosome and desmosomal morphogenesis during epidermal wound healing. *Journal of Ultrastructure Research*, **45**, 93.

ORWIN, D.F.G., THOMSON, R.W. & FLOWER, N.E. (1973) Plasma membrane differentiations of keratinising cells of the wool follicle. *Journal of Ultrastructure Research*, **45**, 1.

WOLFF, K. (1973) Melanocyte–keratinocyte interactions *in vivo*: the fate of the melanosomes. *Yale Journal of Biology and Medicine*, **46**, 384.

British Journal of Dermatology (1976) **95**, 513.

The epidermis in thyroid disease*

P.J.A.HOLT, J.LAZARUS AND R.MARKS

Department of Medicine, Welsh National School of Medicine,
Heath Park, Cardiff CF4 4XW

Accepted for publication 2 April 1976

SUMMARY

Epidermal dimensions, replication and anabolic activity have been studied in thirteen patients with thyrotoxicosis and seven patients with hypothyroidism. Epidermal thickness and rete pattern were significantly reduced in hypothyroidism. The rates of epidermal cell division and anabolic activity in the epidermis were estimated by measuring the rates of incorporation of tritiated precursor compounds and were found to be increased in thyrotoxicosis. The mean autoradiographic labelling indices after the intracutaneous injection of tritiated thymidine were 9·1% in thyrotoxic patients and 4·3% in the hypothyroid group. None of the changes observed could be correlated accurately with the degree of thyroid gland activity, but increased thyroid activity was reflected in the epidermal changes better than decreased thyroid activity.

It is well known that changes in the skin are not infrequently present in patients with thyroid disease. In thyrotoxicosis the skin feels warm and smooth and there is often increased sweating and pigmentation. In hypothyroidism the hair becomes coarse and the dermis thickens (Black, Bottoms & Shuster, 1972). In addition, the skin is dry and sometimes scaly, changes which seem to be essentially epidermal in origin.

The aim of this study was to determine whether epidermal dimensions, growth and anabolic activity are altered in thyroid disease, and secondly to determine whether there is a correlation between any of the changes observed and thyroid gland activity.

PATIENTS AND METHODS

Thirteen patients with thyrotoxicosis (8 female, 5 male, aged 29–78 years, mean 53 years) and seven patients with hypothyroidism (6 female, 1 male, aged 60–83 years, mean 68 years) were studied and compared to a control group of twenty-five normal adults (17 female, 8 male aged 18–77 years, mean 51 years). A diagnosis of thyroid disease was made by clinical examination and confirmed by estimation of serum thyroxine iodine and a free thyroxine index. In eight patients, serum tri-iodothyronine levels were estimated.

---

* Based on a paper read at the Meeting of the European Society for Dermatological Research and the Society of Investigative Dermatology, Amsterdam, June 1975.

Epidermal dimensions

Skin biopsies were taken from the deltoid area in all patients studied. The tissue was fixed in 10% formalin, dehydrated, and embedded in paraffin before sectioning and staining with haematoxylin and eosin.

(i) The number of cell layers forming the viable epidermal compartment was determined by direct counting of cells from the basal layer to the top of the granular cell layer along an axis perpendicular to the skin surface at thirty random points and expressed as the 'mean epidermal cell number' (MECN).

(ii) The rete pattern of the epidermis was measured by calculating the ratio of the length of the basal layer to the length of the top of the granular cell layer (B/G ratio) using a microscope drawing tube and projection technique.

(iii) The size of the viable epidermal cells was expressed as the mean epidermal cell diameter (MECS). This was calculated by dividing the actual thickness of the viable epidermis in microns by the MECN. Actual epidermal thickness was measured at thirty points using a calibrated eyepiece graticule.

Epidermal growth

The replicative activity of the epidermis was studied in two ways.

In vivo *method.* This was performed in fifteen patients over 40 years of age. Biopsies were taken from the deltoid area 1 h following intracutaneous injection of 10 μCi of tritiated thymidine (specific activity of 18 Ci/mmol) in 0·1 ml normal saline. Autoradiographs were prepared using a dipping method (Marks & Wells, 1973). The numbers of basal and suprabasal cells that became labelled in DNA synthesis were expressed as a percentage of the total number of basal cells (labelling index LI), a minimum of 1000 being counted.

In vitro *method.* The rates of incorporation of tritiated thymidine into skin sheets were estimated (see below). Results were expressed in corrected counts per min/per mm^2 of skin/h incubation (thymidine units).

Anabolic activity

Anabolic activity of the epidermis was measured in thirteen of the twenty patients with thyroid disease and a control group of fifteen normal adults using a method similar to that of Marks, Fukui & Halprin (1971) by estimating the rates of incorporation of tritiated proline (specific activity 18 Ci/mmol) and histidine (specific activity 44 Ci/mmol) into pieces of skin 0·4 mm thick and approximately 50 mm^2 in area which had been removed from the lateral aspect of the thigh using a Castroviejo keratome. The skin portions were incubated with the epidermis uppermost in Eagle's minimal essential medium for 4 h at 37°C in an atmosphere of 95% air and 5%CO_2. The radioactive precursors were added to the medium in a concentration of 1 μCi/ml medium. Tritiated thymidine (specific activity 2 Ci/mmol) was used to assess replicative activity (see *in vitro* method above). After incubation the skin was washed, measured, homogenized and extracted with perchloric acid. The residue was solubilised in a highly basic biological solvent (Soluene-Packard) and the incorporated radioactivity was measured in a scintillation counter. The results were expressed as corrected counts per min/mm^2 of skin/h incubation (proline or histidine units).

RESULTS

The results are shown in Table 1. It can be seen that the B/G ratio, MECN and epidermal thickness are reduced significantly in hypothyroidism compared to the control group. No significant correlations

TABLE 1. Epidermal dimensions, labelling indices and anabolic activity in the thyrotoxic, hypothyroid and control groups (Mean ± s.e. mean) and statistical significance of the differences between the measurements in the thyrotoxic or hypothyroid groups and the control group. Direct comparison between the measurements of the thyrotoxic and hypothyroid groups showed differences which were statistically significant at or below the 5% level in all parameters except MECS and thymidine incorporation

	Thyrotoxicosis	Control	Hypothyroidism
B/G ratio	1·14 ± 0·06 n.s.	1·12 ± 0·02	1·04 ± 0·04 $0{\cdot}05 > P > 0{\cdot}025$
MECN	4·7 ± 0·2 n.s.	4·5 ± 0·2	2·9 ± 0·1 $P < 0{\cdot}01$
Epidermal thickness (µm)	54·34 ± 3·18 n.s.	50·43 ± 3·06	35·15 ± 4·57 $0{\cdot}01 > P > 0{\cdot}005$
MECS (µm)	11·98 ± 0·4 n.s.	11·40 ± 0·4	11·95 ± 1·26 n.s.
LI%	9·1 ± 0·8 $P < 0{\cdot}001$	5·1 ± 0·4	4·3 ± 0·6 n.s.
Proline incorporation (proline units)	142·2 ± 23·5 $0{\cdot}005 > P > 0{\cdot}001$	59·7 ± 7·4	37·1 ± 12·9 n.s.
Histidine incorporation (histidine units)	35·9 ± 5·6 $0{\cdot}05 > P > 0{\cdot}025$	22·1 ± 2·5	20·1 ± 7·5 n.s.
Thymidine incorporation (thymidine units)	21·1 ± 3·1 n.s.	15·1 ± 2·3	14·9 ± 5·1 n.s.

n.s., not significant.

existed between the measurements of the B/G ratio and MECN and serum thyroxine iodine, but there was a positive correlation ($r = 0{\cdot}8$) (Fig. 1) between MECN and serum tri-iodothyronine in the thyrotoxic group. These changes in epidermal architecture in thyroid disease are shown in Fig. 2.

Although there was a significantly increased labelling index and significantly increased proline and histidine incorporation in the thyrotoxic group there was no significant correlation between these measurements and serum thyroxine iodine. Fig. 3 is an autoradiograph of skin taken from a patient with thyrotoxicosis.

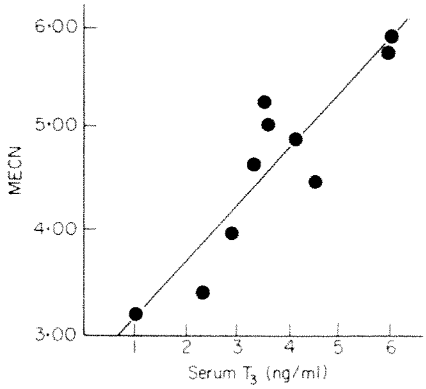


FIGURE 1. Correlation between MECN and serum tri-iodothyronine (T3). $r = +0{\cdot}796$; $0{\cdot}05 > P > 0{\cdot}01$.

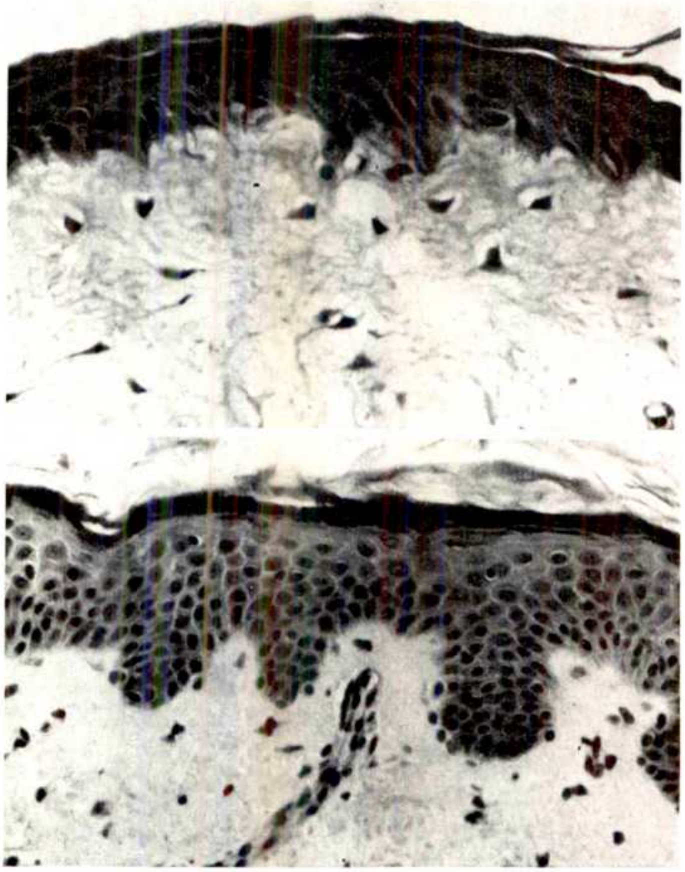


FIGURE 2. Photomicrograph of skin from deltoid area in (top) patient with hypothyroidism illustrating the featureless rete pattern and epidermal thinness contrasting with (bottom) the skin in thyrotoxicosis showing a more pronounced rete pattern and greater thickness of the viable epidermis (H & E, × 90).

DISCUSSION

The significantly increased epidermal labelling index found in thyrotoxicosis together with the more pronounced rete pattern of the epidermis contrasts with the low labelling index and B/G ratio in hypothyroidism and strongly suggests that increased thyroid gland activity is associated with increased epidermal cell proliferation. The use of 'labelling indices' after exposure to tritiated thymidine is commonly held to reflect the proliferative status of the epidermis. This is not strictly correct as this measurement will also record changes in the length of the DNA synthesis period (Weinstein & Frost, 1969). Unfortunately practical considerations do not allow the use of 'labelled mitoses' or 'double labelling' methods for determination of the length of each phase of the mitotic cycle in a group of patients. Despite these considerations it should be said that the 'labelling index' still provides a useful measure of epidermopoiesis as in these disorders in which the length of 'S' phase has been measured it has not differed markedly from normal. Although the enhanced rate of epidermal cell proliferation in psoriasis (Porter & Shuster, 1968; Weinstein & Frost, 1969) and lichen simplex (Marks & Wells, 1973) is well known, there appear to be no reported instances of systemic disease affecting epidermopoiesis.

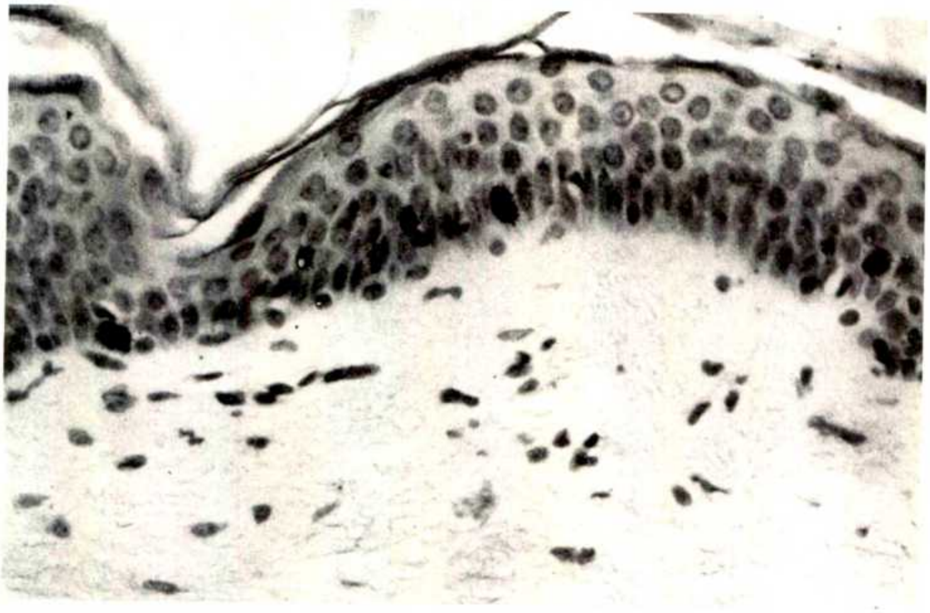


FIGURE 3. Autoradiograph of skin taken from a patient with thyrotoxicosis (H & E, × 150).

The tritiated precursors proline and histidine are incorporated into epidermal proteins which are continuously formed in maturing epidermis and then destroyed or incorporated into proteins which are stable and appear in surface scales. Histidine is preferentially incorporated in the granular cell layer of the epidermis during keratinization (Fukuyama & Epstein, 1966). Incorporation of these amino acids is increased by between 50 and 150% in thyrotoxicosis and suggests that epidermal anabolism is also enhanced in this disorder.

Collagen metabolism is altered in thyrotoxicosis with both an increased breakdown of collagen (Kivirriko *et al.*, 1964) and an increase in collagen synthesis (Gries & Lindner, 1965). However, it should be noted that proline incorporation into our skin specimens is for the most part epidermal and not dermal. The dermal component of the keratome specimen occupies less than 15% of the total thickness of the specimen and is made up of comparatively hypocellular material. Autoradiographs of skin incubated in the presence of tritiated proline demonstrate that the bulk of the incorporation is in fact epidermal (Marks, 1975a). If the incorporations of histidine and proline are corrected for the altered epidermal thickness, the results show the same trend, incorporation in thyrotoxicosis remaining significantly elevated in comparison to the control and hypothyroid group. Lack of precise information concerning precursor pool size and possible alteration of this value in thyroid disease prohibit us from deducing from the altered rates of incorporation that there are altered rates of synthesis of macromolecules. However, because the amount of precursor added is comparatively large (Smets, 1969) it appears probable that protein synthesis parallels amino acid incorporation.

The results of the *in vitro* tests of thymidine incorporation apparently show a less dramatic trend than those obtained by autoradiography from the *in vivo* studies. This apparent discrepancy may be due to the fact that the *in vivo* method is a measure of the number of cells in DNA synthesis whereas the *in vitro* method we have used is a measure of the rate of incorporation of thymidine into skin sheets and may also reflect nuclear DNA repair and extranuclear DNA synthesis. A further contributory fact is that thymidine appears to diffuse less readily through intact tissue *in vitro* compared to its diffusion *in vivo*.

It is perhaps surprising that proline and histidine incorporation did not correlate well with thyroid gland activity. This may be because thyroxine is only one of several hormones influencing epidermopoiesis. We have shown that epidermopoiesis is increased in acromegaly and growth hormone may be responsible for this (Holt & Marks, 1976). In addition corticosteroids are well known to affect profoundly epidermal cell production (Marks & Williams, 1975). Alternatively there may be a level of thyroid hormone above or below which the factors controlling cell size and replication cease to exert a further effect.

Increased rates of cell proliferation and heightened anabolic activity have been demonstrated in relatively few other tissues in thyroid disease. The bone marrow was shown by Axelrod & Berman (1951) to become hypercellular in thyrotoxicosis with hyperplasia of the myeloid series whereas in hypothyroidism the reverse effect occurred (Axelrod & Berman, 1951; Tudhope & Wilson, 1960). A further example is the decreased replication in duodenal mucosa of rats following thyroidectomy (Leblond & Carriere, 1955).

It is of interest that the rate of epidermal cell production can be increased by approximately 100% without much (if any) macroscopic change in the skin. Although there is a rough overall relationship between the clinical morphology of an area of skin and its rate of epidermopoiesis the relationship is a very tenuous one (Marks, 1975b).

ACKNOWLEDGMENT

R.M. is in receipt of a grant from the Wellcome Trust.

REFERENCES

AXELROD, A.R. & BERMAN, L. (1951) Bone marrow in hyperthyroidism and hypothyroidism. *Blood*, **6**, 436.

BLACK, M.M., BOTTOMS, E. & SHUSTER, S. (1972) Skin collagen and thickness in hyperthyroidism and myxoedema. *Clinical Endocrinology*, **1**, 253.

FUKUYAMA, K. & EPSTEIN, W.L. (1966) Epidermal keratinization: localization of isotopically labelled amino acids. *Journal of Investigative Dermatology*, **47**, 551.

GRIES, G. & LINDNER, J. (1965) Influence of thyroid function on connective tissue: clinical and experimental results. *Acta Endocrinologica*, Supplement **100**, 190.

HOLT, P.J.A. & MARKS, R. (1976) Epidermal structure and metabolic activity in acromegaly. *British Medical Journal*, **i**, 496.

KIVIRRIKO, K.E., KOIVUSALO, M., LAITINEN, O. & LAMBERG, B.A. (1964) Hydroxyproline in the serum and urine of patients with hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism*, **24**, 222.

LEBLOND, C.P. & CARRIERE, R. (1955) The effect of growth hormone and thyroxine on the mitotic rate of intestinal mucosa of the rat. *Endocrinology*, **56**, 261.

MARKS, R. (1975a) Increased DNA and protein synthesis in the involved and uninvolved skin of patients with psoriasis. *British Journal of Dermatology*, **93**, Supplement **11**, 28.

MARKS, R. (1975b) Is there a relationship between clinical morphology and epidermal cell kinetics? *Proceedings of the Royal Society of Medicine*, **68**, 161.

MARKS, R., FUKUI, K. & HALPRIN, K. (1971) The application of an *in vitro* technique to the study of epidermal replication and metabolism. *British Journal of Dermatology*, **84**, 453.

MARKS, R. & WELLS, G.C. (1973) Lichen simplex: morphodynamic correlates. *British Journal of Dermatology*, **88**, 249.

MARKS, R. & WILLIAMS, K. (1976) The action of topical corticosteroids on the epidermal cell cycle. *Symposium on Mechanisms of Topical Corticosteroid Activity*. Churchill Livingstone (in press).

PORTER, D. & SHUSTER, S. (1968) Epidermal renewal and amino acids in psoriasis and pityriasis rubra pilaris. *Archives of Dermatology*, **98**, 339.

SMETS, L.A. (1969) Discrepancies between precursor uptake and DNA synthesis in mammalian cells. *Journal of Cell Physiology*, **74**, 63.

TUDHOPE, G.R. & WILSON, G.M. (1960) Anaemia in hypothyroidism. *Quarterly Journal of Medicine*, **29**, 513.

WEINSTEIN, G.D., FROST, P. (1969) Cell proliferation kinetics in benign and malignant skin disease in humans. *National Cancer Institute Monograph*, **30**, 225.

British Journal of Dermatology (1976) **95**, 519.

Plasma volume, intravascular albumin and its transcapillary escape rate in patients with extensive skin disease

HANS-HENRIK PARVING, ANNE-MARIE WORM AND NIELS ROSSING

Department of Medicine A, Rigshospitalet, University of Copenhagen, Department of Clinical Physiology, Bispebjerg Hospital, and The Departments of Dermatology and Clinical Physiology, The Finsen Institute, Copenhagen, Denmark

Accepted for publication 21 April 1976

SUMMARY

Plasma volume and plasma concentration and transcapillary escape rate of albumin (TER_{alb}), i.e. the fraction of intravascular mass of albumin that passes to the extravascular space per unit time, were determined using ^{125}I-labelled human albumin in eight patients with extensive skin disease. Plasma volume and plasma albumin concentration were reduced ($P<0{\cdot}05$). Thus the intravascular albumin mass was moderately decreased to an average of $0{\cdot}55 \pm 0{\cdot}06$ (s.d.) g/cm height compared with a normal mean value of $0{\cdot}77 \pm 0{\cdot}07$ (s.d.) g/cm. This 29% decrease is statistically significant ($P<0{\cdot}001$). The transcapillary escape rate of albumin (TER_{alb}) was significantly elevated, mean $8{\cdot}6 \pm 1{\cdot}1$ (s.d.) $\% \times h^{-1}$, as compared to normal subjects, mean $5{\cdot}6 \pm 1{\cdot}1$ (s.d.) $\% \times h^{-1}$, (+54%, $P<0{\cdot}001$). The same patients were studied again after a 1-week treatment with prednisone, 25–60 mg per day. Plasma albumin concentration, plasma volume and intravascular mass of albumin were unchanged, while TER_{alb} decreased significantly during treatment, mean $5{\cdot}9 \pm 0{\cdot}8$ (s.d.)$\% \times h^{-1}$, ($P<0{\cdot}01$).

It is suggested that displacement of albumin into the skin and loss of albumin from the skin are the dominating mechanisms of the reduction in the intravascular albumin mass in patients with extensive skin disease.

Decreased plasma albumin concentration is a regular feature of extensive skin disease (Tickner & Basit, 1960; Shuster & Wilkinson, 1963; Shuster & Marks, 1970). Shuster & Marks (1970) in a recent review suggested that plasma volume expansion was the most important cause. In order to evaluate this we measured plasma volume and intravascular albumin mass in this type of patient. Since displacement of albumin into the skin might well be at work—as demonstrated by others in severe burns (Birke, Liljedahl & Plantin, 1968)—we measured the transcapillary escape rate of albumin (TER_{alb}), i.e. the fraction of intravascular mass of albumin that passes to the extravascular space per

---

Correspondence: Dr H.-H. Parving, Department of Clinical Physiology, Bispebjerg Hospital, Bispebjerg Bakke, 2400 Copenhagen N.V., Denmark.

TABLE I. Clinical data in patients with extensive skin disease

Subject	Sex	Age (years)	Height (cm)	Weight (kg)	Surface area (m²)	Diagnosis	Extent of skin disease	Prednisone (mg/day)	Clinical effect of therapy
1	F	79	158	67	1·68	Erythroderma	Generalized	30	+
2	M	60	165	59	1·65	Erythroderma	Generalized	40	+ +
3	M	62	169	73	1·82	Erythroderma	Generalized	30	+
4	M	70	167	65	1·72	Erythroderma	Generalized	25	+ +
5	M	69	170	62	1·72	Erythroderma	Generalized	25	+
6	M	79	178	64	1·79	Photodermatitis	40*	30	+ +
7	M	69	182	100	2·20	Bullous pemphigoid	20*	60	+ + +
8	F	68	160	72	1·75	Contact dermatitis	75*	30	+ + +
Mean ± s.d.		69·4	169	70·2	1·79				
		6·9	8·2	12·9	0·17				
†Mean ± s.d.		50·7	175	76·0	1·90				
		5·8	4·5	7·8	0·12				

* Involved skin area (%), estimated by the 'rule of nines'; † Values from Parving & Gyntelberg (1973) for 10 male subjects.

unit time. This was done with ^{125}I-labelled human albumin. The effect on the above mentioned variables of systemic corticosteroid treatment was also studied.

MATERIALS AND METHODS

Eight patients with extensive skin disease were investigated. Clinical data are presented in Table 1. All patients had normal chest X-ray and ECG; clinical signs of cardiac failure were not present in any of the patients (the jugular venous pressure was not determined). None had hypertension, diabetes mellitus, or any other major medical or surgical disease apart from their skin disease. The urine was without protein as tested with Albustix. At the time of the first investigation only bland topical treatment was applied. Then prednisone was given (25–60 mg daily), and the second investigation was carried out about 1 week later.

The investigative procedure and the theoretical basis for the calculation of the transcapillary escape rate of albumin (TER_{alb}) from the initial disappearance rate of labelled plasma albumin have been described in detail previously (Parving & Gyntelberg, 1973; Parving, 1975). The patients were studied in the morning after at least 12 h of fasting and 30 min of rest in the supine position; human serum albumin labelled electrolytically with ^{125}I (code MIAK, Institute for Atomic Energy, Kjeller, Norway) was used. This tracer preparation contains less than 1·0% free radioactive iodide and has, by metabolic studies, been demonstrated to behave like endogenous albumin (Rossing & Jensen, 1967; Parving, Rossing & Jensen, 1974). About 5 μCi of the tracer was used in the first investigation and about 20 μCi in the second. The tracer was injected into one arm vein, and nine venous blood samples of 6 ml each were drawn from the other arm (10, 15, 20, 30, 35, 40, 50, 55, and 60 min after injection). TER_{alb} was determined as the rate constant of the practically monoexponential decrease in specific plasma activity over the first 60 min after injection as calculated by the least square method.

Plasma volume (PV) was determined by extrapolation to time zero of the plasma disappearance curve of tracer albumin, and the injected volume of tracer measured by weighing. Plasma albumin concentration was measured according to Laurell (1966). Intravascular mass of albumin (IVM) equals plasma volume × plasma albumin concentration.

The results were compared with those obtained in ten normal adults by exactly the same techniques (Parving & Gyntelberg, 1973).

Statistical analysis was performed using the Wilcoxon's test for paired and unpaired observations.

RESULTS

Table 2 shows the measured variables before and during prednisone treatment. TER_{alb} was markedly increased at the first investigation. The average was $8{\cdot}6 \pm 1{\cdot}1$ (s.d.)% $IVM \times h^{-1}$, compared with a normal average of $5{\cdot}6 \pm 1{\cdot}1$ (s.d.)% $IVM \times h^{-1}$, ($P < 0{\cdot}001$). The prednisone treatment caused a significant 46% reduction in TER_{alb} to an average value of $5{\cdot}9 \pm 0{\cdot}8$ (s.d.)% $IVM \times h^{-1}$, ($P < 0{\cdot}01$). This value did not differ statistically from the normal control values ($P > 0{\cdot}10$). Plasma albumin concentrations, PV and IVM did not differ statistically significantly at the two investigations. Table 3 demonstrates that plasma albumin concentration, PV and IVM were significantly reduced as compared with the normal male values of Parving & Gyntelberg (1973). Exclusion of the two female patients (Nos 1 and 8) did not alter this fact. The reduction was significant whether the values were expressed in relation to body weight (except PV), height or surface area, $P < 0{\cdot}05$. Plasma volume was higher and close to normal values in the five patients with erythroderma than in those with other skin diseases. IVM was reduced to about the same degree in both groups of patients.

TABLE 2. Transcapillary escape rate of albumin, albumin, plasma volume and intravascular albumin mass before (I) and during prednisone treatment (II) of patients with extensive skin disease

Subject	TER$_{alb}$ (% IVM × h^{-1})		Albumin (g/litre)		Plasma volume (ml)		Intravascular albumin mass (g)	
	I	II	I	II	I	II	I	II
1	6·7	6·4	29·0	30·1	3020	2860	88	86
2	10·1	6·0	26·8	26·8	2860	2780	76	75
3	8·6	6·6	34·1	30·7	2760	2940	94	90
4	8·1	6·6	28·0	28·4	3410	3490	95	99
5	8·6	5·5	35·5	32·9	3060	3350	109	110
6	9·9	6·2	32·8	29·2	2640	2740	87	80
7	8·1	5·6	35·5	36·5	2650	2860	94	101
8	8·4	4·3	36·4	31·2	2730	3050	99	95
Mean	8·6	5·9	32·3	30·7	2891	3009	93	92
± s.d.	1·1	0·8	3·9	3·0	262	273	10	11
P	< 0·01		n.s.		n.s.		n.s.	

TABLE 3. Albumin, plasma volume and intravascular mass of albumin in extensive skin disease

Subject	Albumin† (g/litre)	Plasma volume†				Intravascular albumin mass†			
		(ml)	(ml/kg)	(ml/cm)	(litre/m^2)	(g)	(g/kg)	(g/cm)	(g/m^2)
1	29·0	3020	45·3	19·2	1·80	88	1·31	0·56	52·4
2	26·8	2860	48·5	17·3	1·73	76	1·29	0·46	46·0
3	34·1	2760	37·8	16·3	1·52	94	1·29	0·56	51·7
4	28·0	3410	52·4	20·4	1·98	95	1·46	0·57	55·2
5	35·5	3060	49·5	18·0	1·78	109	1·76	0·64	63·5
6	32·8	2640	41·3	14·8	1·47	87	1·36	0·49	48·6
7	35·5	2650	26·5	14·6	1·20	94	0·94	0·52	42·7
8	36·4	2730	38·0	17·1	1·56	99	1·37	0·62	56·6
Mean ± s.d.	32·3	2891	42·4	17·2	1·63	93	1·35	0·55	52·0
	3·8	262	8·4	2·0	0·24	10	0·23	0·06	6·5
Mean* ± s.d.	37·8	3562	48·3	20·3	1·88	135	1·83	0·77	71·0
	2·0	344	6·7	1·7	0·19	15	0·26	0·07	7·0
P	< 0·005	< 0·001	< N.S.	< 0·005	< 0·05	< 0·001	< 0·001	< 0·001	< 0·001

* Values from Parving and Gyntelberg (1973) for 10 male subjects; † values obtained before prednisone treatment.

DISCUSSION

Initially, it should be mentioned that our male control group is younger than the patients studied. However, this is without importance with regard to the TER values and plasma albumin concentrations, since these variables in adults are largely age independent (Parving, 1975; Weeke & Krasilnikoff, 1972). While the individual plasma volume expressed in absolute terms is relatively constant during adult life (Chien *et al.*, 1966; Sjöstrand, 1963), plasma volume expressed in relation to body weight is age dependent due to increasing body weight with age (Sjöstrand, 1963). This disturbing influence can be avoided by expressing plasma volume and IVM in relation to body height as done in the present study.

The finding of an increased TER_{alb} in patients with extensive skin disease is in agreement with the findings of Marks & Shuster (1966) in nine patients with erythroderma and three with other extensive skin disease. Quantitatively, however, the results differ markedly since these authors reported an average TER_{alb} that can be calculated to be 115% IVM × h^{-1} (range 10·8–630% IVM × h^{-1}). In other words, their technique led them to suggest that on average somewhat more than the whole circulating albumin mass left the bloodstream through the microvascular wall per hour, and that this figure could increase about sixfold in extreme cases. In sharp contrast we found that only about one-twelfth of the intravascular albumin mass escaped circulation per hour (8·5% IVM × h^{-1}). Therefore all crucial elements of the investigative procedures should be critically analyzed before the results are compared and interpreted. The paramount prerequisite for correct results regarding the endogenous TER_{alb} is that the organism does not distinguish between labelled and endogenous albumin. In a recent detailed discussion on the metabolic properties of the tracer it was concluded that its metabolic behaviour was satisfactory (Parving *et al.*, 1974). It is now known that albumin prepared by fractionation in an alcohol ether system, as used by Marks & Shuster (1966), causes an unpredictable degree of denaturation of the protein and invalidates its tracer properties. In addition the amount of free ^{131}I reached 5% in their product. Both factors give rise to an overestimation of the TER_{alb} and PV, and to an increase in the variation of the measurements. This conclusion is further validated by their finding of a TER_{alb} value in control subjects of 17·4% IVM × h^{-1}, which is much higher than those reported in other studies (for review see Parving, 1975).

Even though the protein excretion in the urine and in the gut has been found slightly increased in some patients with extensive skin disease (Trotter & Fairburn, 1967; Cotterill, Cunliffe & Bulusu, 1971; Shuster & Marks, 1970), the contribution from these routes of protein loss constitutes maximally a few percent of the overall albumin extravasation rate. Thus it can be concluded that the increased TER_{alb} reflects increased albumin extravasation in the skin, probably due to increased microvascular permeability. It has not been measured previously, to what extent corticosteroids normalize this leakage. However, this effect is the probable rationale of their clinical use against local or more generalized transudative or exudative processes of allergic and inflammatory conditions, in burns, and possibly also in conditions with increased intracerebral pressure. The mode of action of corticosteroids is unknown. The effect of corticosteroids may be directly on the endothelial membrane narrowing the intercellular gaps or—more likely—may be a neutralizing effect on vasoactive substances as for example histamine and bradykinin released in abnormal amounts in these pathological conditions.

Hypoalbuminaemia, as demonstrated in the present study, is nearly always present in patients with extensive skin disease (Shuster & Marks, 1970). The possible explanations are: (1) decreased rate of synthesis, (2) increased endogenous catabolism, (3) external albumin loss, (4) a non-steady displacement of albumin from the intra- to the extravascular space, and finally (5) plasma volume expansion. Shuster & Wilkinson (1963) studied the albumin metabolism in patients with extensive skin disease and found the rates of albumin synthesis and breakdown about normal. They suggested that plasma volume expansion was the most important factor in the production of hypoalbuminaemia, since they found the product of PV and plasma albumin concentration to be normal.

Increased plasma volume has also been found in later studies by the same group (Marks & Shuster, 1966; Fox *et al.*, 1965). These results are in clear contrast to the present findings of a slightly reduced plasma volume and a more pronounced decrease in intravascular albumin mass. Some of these discrepancies have already been discussed above. While the plasma volume was determined by extrapolation to time zero in the present study, the above mentioned authors used tracer dilution 10 min after the intravenous injection. This will always lead to an overestimation of plasma volume, particularly in conditions with increased albumin extravasation.

Actually, with a better technique, namely ^{51}Cr-erythrocytes and haematocrit determinations, Marks & Shuster (1966) themselves had obtained normal plasma volumes. A slightly reduced plasma volume and a more pronounced decrease in intravascular albumin mass were found in the present study. On this background it seems fair to conclude that plasma volume is normal or slightly reduced, and the intravascular albumin mass is reduced in patients with extensive skin disease. Since the rate of synthesis and the endogenous catabolism of albumin have been found about normal (Shuster & Wilkinson, 1963), the reduced intravascular albumin mass must be due to external loss from the skin following displacement of albumin from the circulation into the skin as reflected by the increased TER_{alb} and the finding of a relative increase in the extravascular compared to the intravascular albumin mass (Shuster & Wilkinson, 1963). From a kinetic point of view, this explanation only holds true if it can be assumed that the patients are in metabolically non-steady state. Since the patients are acutely ill, this assumption is probably fulfilled. However, the normalization of TER_{alb} as measured after 1 week of steroid treatment had in no way brought the circulating albumin mass back to normal. This warrants renewed direct metabolic measurements of the rate of synthesis and the survival of albumin.

In this context it should be mentioned that the reduced plasma albumin concentration, plasma volume and intravascular albumin mass are all regular findings in extensive burns—a condition that in many ways simulates severe skin disease. Here the reduced intravascular mass of albumin is also due to displacement of albumin from the intravascular to the extravascular space and to loss of albumin from the skin (Birke *et al.*, 1968). It is natural to consider the intravascular to extravascular shift of macromolecular plasma substances to be an important factor in the oedema associated with severe skin disease and burns.

ACKNOWLEDGMENT

We wish to thank Hanne-Marie Heidelbach for skilful technical assistance.

REFERENCES

BIRKE, G., LILJEDAHL, S.-O. & PLANTIN, L.-O. (1968) Distribution and losses of plasma proteins during the early stage of severe burns. *Annals of the New York Academy of Sciences*, **150**, 895.

CHIEN, S., USAMI, S., SIMMONS, R.L., MCALLISTER, F.F. & GREGERSEN, M. (1966) Blood volume and age: repeated measurements on normal men after 17 years. *Journal of Applied Physiology*, **21**, 583.

COTTERILL, J.A., CUNLIFFE, W.J. & BULUSU, L. (1971) Proteinuria associated with skin disease. *Lancet*, **ii**, 403.

FOX, R.H., SHUSTER, S., WILLIAMS, R., MARKS, J., GOLDSMITH, R. & CANDON, R.E. (1965) Cardiovascular, metabolic and thermoregulatory disturbances in patients with erythrodermic skin disease. *British Medical Journal*, **i**, 619.

LAURELL, C.-B. (1966) Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analytical Biochemistry*, **15**, 45.

MARKS, J. & SHUSTER, S. (1966) Method for measuring capillary permeability and its use in patients with skin disease. *British Medical Journal*, **ii**, 88.

PARVING, H.-H. (1975) Microvascular permeability to plasma proteins in hypertension and diabetes mellitus in man—on the pathogenesis of hypertensive and diabetic microangiopathy. *Danish Medical Bulletin*, **22**, 217.

PARVING, H.-H. & GYNTELBERG, F. (1973) Transcapillary escape rate of albumin and plasma volume in essential hypertension. *Circulation Research*, **32**, 643.

PARVING, H.-H., ROSSING, N. & JENSEN, H.Æ. (1974) Increased metabolic turnover rate and transcapillary escape rate of albumin in essential hypertension. *Circulation Research*, **35**, 544.

ROSSING, N. & JENSEN, H. (1967) Metabolism of different albumin preparations. *Clinical Science*, **32**, 89.

SHUSTER, S. & MARKS, J. (1970) Protein metabolism. In: *Systemic effects of skin disease*, p. 52. Heinemann, London.

SHUSTER, S. & WILKINSON, P. (1963) Protein metabolism in exfoliative dermatitis and erythroderma. *British Journal of Dermatology*, **75**, 344.

SJÖSTRAND, T. (1963) Blood volume. In: *Handbook of Physiology* (Ed. by W.F. Hamilton and P. Dow), section 2, vol. 2, p. 51. Waverly Press, Baltimore.

TICKNER, A. & BASIT, A. (1960) Serum proteins and liver function in exfoliative dermatitis. *British Journal of Dermatology*, **72**, 138.

TROTTER, M.D. & FAIRBURN, E.A. (1967) Subclinical proteinuria in dermatological outpatients. *Lancet*, **ii**, 231.

WEEKE, B. & KRASILNIKOFF, P.A. (1972) The concentration of 21 serum proteins in normal children and adults. *Acta medica Scandinavica*, **192**, 149.

British Journal of Dermatology (1976) **95**, 525.

Physical training and connective tissues in young mice

BIOCHEMISTRY OF SKIN

A. KIISKINEN AND E. HEIKKINEN

Department of Public Health, University of Jyväskylä, Finland

Accepted for publication 13 April 1976

SUMMARY

The effect of physical training on some connective tissue components of the skin was studied in male mice of NMRI-strain. The mice to be trained and their controls were about 2-weeks-old at the beginning of the training, which took place on a 5° inclined treadmill 5 days a week for 3–22 weeks progressively. The final exercise bouts were 50 and 80 min for moderate programmes and 180 min for the intensive programme at a speed of 30 cm/s. At the end of each experiment skin samples from trained and control animals were taken for the analyses.

We found increased concentrations of nitrogen, hexosamines and hydroxyproline in the skin of the trained mice compared to the controls. The unchanged DNA concentration suggests that the activation of synthesis occurred in a constant number of cells.

The effect of physical training on the connective tissue of the skin has not so far been the subject of scientific publications with the exception of the report by Heikkinen & Vuori (1972). They observed a slightly retarded hydroxyproline metabolism in the skin of aged inactive mice, whereas physically active aged mice had higher concentrations of nitrogen and hexosamines in the skin.

The results prompted the questions (a) whether skin taken from physically active mice shows the above-mentioned changes if training is started early during the growth period, (b) whether the metabolic changes occur in a constant or altered number of cells, (c) whether the quality and the quantity of the changes depend on the duration and intensity of physical training programs. Thus, we compared the concentrations of nitrogen, hydroxyproline, hexosamines, DNA, and RNA-ribose in the skin samples taken from untrained mice with skin samples of mice which were trained either early during the growth period only or continuously to maturity. The methods and results of simultaneous research concerning the connective tissues of long bones, tendons, and heart will appear in other reports.

MATERIAL AND METHODS

Animals and physical training. A detailed description of the experimental animals and training procedures has been published elsewhere (Kiiskinen, 1976). Two-week-old (14 ± 2 days) male mice of NMRI-strain were randomly assigned to test and control groups. The mice to be trained were gradually adapted to running on a treadmill. The training took place on a 5° inclined treadmill operating at a speed of 30 cm/s 5 days a week for 3–22 weeks. The duration of daily exercise as well as running speed were increased progressively during the first 3 weeks of training. The final daily exercise bouts were 50 and 80 min for moderate training programmes I and II and 180 min for the intensive training programme III. The daily exercise was divided into two sessions, one in the morning and the other in the afternoon.

Preparation of samples. The mice were killed under ether anaesthesia by decapitation. The whole skin was removed at room temperature and immediately chilled in ice in sealed plastic tubes and stored deep-frozen (at –20°C) until preparation. The skin was allowed to thaw at room temperature. Thereafter it was carefully shaved, subcutaneous tissue including muscle was scraped off with a blunt knife

TABLE 1. Effects of 3–22 weeks' moderate training (I + II) on the gain of body weight. Mean ± sd and number of observations are given together with Student's *t*-test

Variable	Training time (weeks)	Animals		*P*
		Trained	Control	
Initial body weight (g)	(3)	11·1 ± 1·6 (33)	11·0 ± 1·6 (43)	n.s.
	(7)	12·7 ± 2·2 (28)	9·9 ± 1·8 (24)	< 0·001
	(12)	12·8 ± 1·1 (28)	10·4 ± 1·9 (22)	< 0·005
	(22)	11·9 ± 5·5 (85)	12·4 ± 5·4 (63)	n.s.
Final body weight (g)	3	28·4 ± 2·3 (14)	28·4 ± 2·4 (14)	n.s.
	7	34·6 ± 2·1 (8)	33·7 ± 3.2 (8)	n.s.
	12	24·4 ± 3·6 (14)	28·7 ± 3·5 (14)	< 0·005
	22	45·6 ± 2·4 (9)	47·2 ± 1·6 (9)	n.s.

Note: some of the above comparison data are taken from Kiiskinen (1976).

TABLE 2. Effects of 3–12 weeks' intensive training (III) on the gain of body weight. Mean ± sd and number of observations are given together with Student's *t*-test

Variable	Training time (weeks)	Animals		*P*
		Trained	Control	
Initial body weight (g)	(3)	9·3 ± 2·3 (43)	9·6 ± 2·4 (31)	n.s.
	(7)	7·0 ± 1·2 (24)	7·8 ± 0·9 (22)	< 0·05
	(12)	11·5 ± 1·1 (30)	11·8 ± 0·9 (20)	n.s.
Final body weight (g)	3	23·2 ± 2·9 (15)	25·8 ± 2·8 (7)	< 0·025
	7	31·7 ± 2·2 (8)	34·8 ± 4·7 (8)	n.s.
	12	40·7 ± 1·7 (11)	42·0 ± 2·7 (14)	n.s.

Note: some of the above comparison data are taken from Kiiskinen (1976).

and it was divided into two equal halves by cutting along the median line of the back. One half was homogenized by being cut into small pieces, dried (at 95°C overnight), weighed (Mettler H 20 T, Switzerland), and used for the measurements of nitrogen, hydroxyproline and hexosamines. The samples used for nucleic acid analyses were prepared at +4°C. Round pieces 22 mm in diameter were taken from the other half of the skin. The samples were frozen and dried in a lyophilizer (Heto, Denmark) for 2 days, weighed and used for nucleic acids analyses.

Chemical methods. The skin sample was hydrolyzed in 2 N HCl overnight at 103°C for the analysis of hexosamines. Hexosamines were freed from interfering chromogens by means of DOWEX-50 cation exchange resin according to Boas (1953). The *hexosamine* concentration was determined by Blix's (1948) modification of the Elson-Morgan colour method. For the determination of nitrogen and hydroxyproline, hydrolyzing was continued in 6 N HCl at 130°C for 3 h and the hydrolyzate was thereafter evaporated completely. The residues were dissolved in distilled water and filtered. Aliquots were taken to determine the concentration of *hydroxyproline* by Stegeman's colour reaction as modified by Woessner (1961). After combustion of the samples in 8 N sulphuric acid the concentration of *nitrogen* was analysed by the method introduced by Minari & Zilversmith (1963). The DNA and RNA fractions were separated by the method of Schmidt & Thannhäuser as modified by Munro & Fleck (1966). The concentration of *DNA* was determined by Burton's colour reaction (1958) and *RNA-ribose* concentration by Geriotti's colour reaction (1955). The variation coefficients for the chemical methods in this experiment were: nitrogen 6·3%, hydroxyproline 4·9%, hexosamines 4·6%, DNA 2·1%, and RNA-ribose 2·5%. The results are given in relation to the dry weight of sample.

In order to ensure a reliable comparison of the results between the trained and control animals special care was taken to analyze the respective samples of the trained and control animals within the

TABLE 3. Effects of 3–22 weeks' moderate training (I + II) on the concentration of nitrogen, hydroxyproline, hexosamines, DNA and RNA-ribose in the skin

Variable (μg/mgDW)	Training time (weeks)	Animals		P
		Trained	Control	
Nitrogen	3	160·07 ± 26·80 (14)	129·81 ± 36·88 (14)	< 0·001
	12	124·62 ± 15·27 (14)	86·35 ± 16·77 (14)	< 0·001
	22	117·78 ± 7·58 (9)	104·81 ± 10·70 (9)	< 0·02
Hydroxyproline	3	76·41 ± 16·58 (14)	59·48 ± 11·77 (14)	< 0·01
	12	85·58 ± 16·13 (14)	61·85 ± 9·79 (14)	< 0·001
	22	80·50 ± 7·96 (9)	85·02 ± 4·93 (9)	n.s.
Hydroxyproline–nitrogen ratio	3	0·48 ± 0·08 (14)	0·59 ± 0·13 (12)	n.s.
	12	0·68 ± 0·08 (14)	0·73 ± 0·12 (14)	n.s.
	22	0·68 ± 0·07 (9)	0·82 ± 0·07 (9)	< 0·005
Hexosamine	3	2·23 ± 0·33 (12)	1·82 ± 0·23 (14)	< 0·001
	12	1·82 ± 0·37 (13)	1·48 ± 0·17 (14)	< 0·01
	22	1·43 ± 0·15 (9)	1·43 ± 0·15 (9)	n.s.
DNA	7	6·61 ± 0·87 (8)	7·26 ± 1·52 (8)	n.s.
RNA-ribose	7	2·61 ± 2·47 (8)	2·71 ± 2·40 (8)	n.s.

Note: mean ± sd and number of observations are given together with Student's *t*-test.

TABLE 4. Effects of 3–12 weeks' intensive training (III) on the concentrations of nitrogen, hydroxyproline, hexosamines, DNA and RNA-ribose in the skin

Variable (μg/mgDW)	Training time (weeks)	Animals		
		Trained	Control	*P*
Nitrogen	3	110·85 ± 24·92 (14)	86·07 ± 11·75 (7)	<0·05
	12	169·73 ± 17·06 (11)	133·21 ± 14·54 (14)	<0·001
Hydroxyproline	3	70·16 ± 19·49 (15)	52·22 ± 8·40 (6)	<0·05
	12	103·19 ± 15·93 (11)	78·04 ± 11·54 (14)	<0·001
Hydroxyproline–nitrogen ratio	3	0·64 ± 0·08 (14)	0·59 ± 0·05 (6)	n.s.
	12	0·61 ± 0·07 (11)	0·59 ± 0·10 (14)	n.s.
Hexosamine	3	2·23 ± 0·27 (15)	1·87 ± 0·33 (7)	<0·001
	12	2·18 ± 0·25 (11)	1·71 ± 0·23 (14)	<0·001
DNA	7	7·15 ± 0·77 (8)	6·96 ± 0·59 (8)	n.s.
RNA-ribose	7	2·54 ± 0·19 (8)	2·20 ± 0·36 (8)	<0·05

Note: mean ± sd and number of observations are given together with Student's *t*-test.

same test series together with the appropriate standards. The comparison of different training programmes and age groups is somewhat impaired by the relatively large variation observed in the initial and final body weights of mice from different litters (Tables 1 and 2) and by some variation in chemical methods over the 3-year experimental period.

Statistical methods. The results were statistically evaluated by Student's *t*-test for non-correlating means (two sided test).

RESULTS

The chemical composition of skin samples in the trained and control mice after 3–22 weeks' moderate and 3–12 weeks' intensive training appear in Tables 3 and 4. The concentrations of nitrogen, hydroxyproline, and hexosamines were increased in the skin samples of the trained animals compared to the control after 3 to 12 weeks' training, whereas the concentration and amount of DNA remained unaffected after both training programmes of 7 weeks. The RNA-ribose concentration increased slightly after 7 weeks' intensive training.

DISCUSSION

The results show that the concentrations of nitrogen and hexosamines increase not only in trained aged mice as observed by Heikkinen and Vuori (1972) but also in mice trained during the growth period. Together with the increased nitrogen and hexosamine concentration we found a parallel increase in the hydroxyproline concentration. The duration of training over the first 12 weeks had no larger influence on the chemical variables. However, when moderate training was extended up to 22 weeks the difference between the groups in hexosamine and hydroxyproline concentration disappeared. The relative amount of hydroxyproline to nitrogen declined after prolonged moderate training. This suggests that moderate physical training may influence the rate of maturation of the skin, e.g. by reducing the accumulation of collagen and by retarding the decrease in ground substance glycosaminoglycans. These changes are characteristic of the ageing process of skin (e.g. Lindner, 1972). The total content or concentration of DNA was not significantly influenced by training. This suggests that the number of cells does not change as a consequence of physical training or that opposite changes in different layers of the skin compensate each other. Physical training appears, however, to activate a constant number of cells to synthesize more collagen and ground substance resulting in a relative increase in these substances compared to DNA. This assumption is also supported by the increased RNA-ribose concentration observed after 7 weeks' intensive training.

Because the connective tissue components were expressed in relation to dry weight and not to surface area of the skin samples, possible changes in other components of skin may also influence the results. Historical analysis as well as the analysis of total lipid content could give more information about such changes due to the structural complexity of skin tissue. The separation of dermis and epidermis is needed also to clarify in which layers of skin chemical adaptation reactions to physical training occur. These results show that the effects of physical training are not limited to the connective tissues of the locomotor system only. This implies that hormonal control mechanisms may play a role in the adaptation of connective tissue to physical training.

ACKNOWLEDGMENT

We wish to thank the Ministry of Education (Research Council for Physical Culture and Sport, Finland) for financial support.

REFERENCES

BLIX, G. (1948) The determination of hexosamines according to Elson and Morgan. *Acta chemica scandinavica*, **2**, 467.

BOAS, N.F. (1953) Method for the determination of hexosamines in tissues. *Journal of biological Chemistry*. **204**, 553.

BURTON, K. (1956) A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochemical Journal*, **62**, 315.

CERIOTTI, G.J. (1955) Determination of nucleic acids in animal tissues. *Journal of biological Chemistry*, **214**, 59.

HEIKKINEN, E. & VUORI, I. (1972) Effect of physical activity on the metabolism of collagen in aged mice. *Acta physiologica scandinavica*, **84**, 543.

KIISKINEN, A. (1976) Physical training and connective tissues in young mice; Physical properties of Achilles tendons and long bones. *Department of Public Health Publication No. 28*, University of Jyväskylä.

LINDNER, J. (1972) Altern des Bindegewebes. In: *Handbuch der allgemeinen Patologie VI/4, Altern* (Ed. by G. Holle) p. 243. Springer, Berlin.

MINARI, O. & ZILVERSMITH, D.B. (1963) Use of KCN for stabilization of color in direct nesslerization of Kjeldahl digests. *Analytical Biochemistry*, **6**, 320.

MUNRO, N. & FLECK, A. (1966) The determination of nucleic acids. In: *Methods of Biochemical Analysis* (Ed. by D.Glick) Vol. 14. p. 113. Wiley Interscience, New York.

WOESSNER, J.F., JR. (1961) The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Archives of Biochemistry*, **93**, 440.

British Journal of Dermatology (1976) **95**, 531.

Localized pemphigoid

JOHN R.PERSON, ROY S.ROGERS III AND HAROLD O.PERRY

The Mayo Clinic and Mayo Foundation
Rochester, Minnesota 55901, U.S.A.

Accepted for publication 30 March 1976

SUMMARY

Of 144 patients with bullous or cicatricial pemphigoid, nine with localized pemphigoid were seen at the Mayo Clinic between 1968 and 1975. In two patients the disease had become generalized before presentation, and in one it had evolved into bullous pemphigoid, the only case with positive indirect immunofluorescence. One additional patient showed mucosal lesions. Direct immunofluorescence was performed in five patients and showed linear deposition of C3 and fibrin but not immunoglobulins in three; in two patients immunofluorescence was negative. Localized pemphigoid can be divided into two types: that with scarring plaque-like lesions usually occurring on the head and neck predominantly in males, and that with localized bullous lesions usually occurring on the lower part of the legs of females.

In his 1953 monograph on pemphigus, Lever noted that the cutaneous lesions in what is now known as cicatricial pemphigoid were of two types: scattered bullae, which heal without scarring, and recurrent cicatricial plaques occurring near mucosal surfaces and on the scalp. In 1957 Brunsting & Perry described seven patients with persistent plaques on the head and neck and associated with bullae and scarring. One patient also had mucosal lesions and all had rare and usually transient bullae or plaques elsewhere on their skin. Their mean age was 56 years. Six patients were atopic and six were males. All biopsies showed subepidermal bullae. At least one patient responded to sulphapyridine but none was exacerbated by iodides. Urinary porphyrins were in the normal range in all patients tested.

In 1958 Földvári reported twenty cases of localized subepidermal bullous eruptions of which only two involved the head, neck, or scalp. Frain-Bell in 1959 reported six cases of acral eczema associated with bullae largely limited to the legs. In at least one, and perhaps two of his cases, the biopsy specimens were consistent with pemphigoid. Eberhartinger & Niebauer (1961) reported the pretibial area to be by far the most common site of involvement and noted that most of their patients were elderly. However, MacVicar & Graham (1966) reported ten cases of localized bullous lesions, which they termed chronic 'localized pemphigoid' and which resembled the cases reported by Brunsting & Perry in that all lesions were located on the head and neck, nine of the ten patients were males, and most of the patients were middle-aged. The response to treatment in general was poor. Two reports have been

---

Reprint requests to: Dr J.R.Person, c/o Section of Publications, Mayo Clinic, 200 First Street SW, Rochester, MN 55901, U.S.A.

published describing the modestly successful results of skin grafting in the treatment of extensive cutaneous ulceration that is rarely associated with cicatricial pemphigoid (Slepyan, Burks & Fox, 1961; Sneddon, 1962). Other isolated cases of localized pemphigoid have been reported (Jonquieres, 1965; Bourlond, Philippart & Hoet, 1970; Stewart *et al.*, 1971), but in only one case, that reported by Trepanier (1970), have immunofluorescent studies been performed. In this instance the patient had only scalp involvement. Biopsy showed eosinophilic papillary micro-abscesses; both direct and indirect immunofluorescence were entirely negative, and the patient responded to sulphones. On the basis of sulphone response, the author termed the case 'probable localized dermatitis herpetiformis'.

TABLE 1. Clinical data on localized and persistent pemphigoid

Age (years) at onset, sex	Clinical involvement	Treatment	Duration	Miscellaneous
63F	R pretibial	Systemic steroids for exacerbations	4 yr at last follow-up (FU)	Varicose veins
29F	Scalp (cicatricial alopecia)	Topical steroids & azathioprine	14 yr at last FU	Pos ANA*
53F	Bilateral pretibial	Topical only	13 months at last FU	No other disease
79M	Neck, forearms	Topical only	1 yr 4 months at last FU	No other disease
75M	Forearms One umbilical bulla	Topical only	Dermatitis for 7 yr; bullae for 13 months but inactive at last FU	Pos ANA
57F	Lower part of legs	Topical; brief course of steroids	5½ yr at last FU	Breast cancer (no recurrence after 10 yr)
77F	Lower part of leg for 20 months Eruption generalized later	Topical only	20 months localized, 2 yr generalized	Died with active disease (cause of death—bronchopneumonia) Arteriosclerosis
58M	Scalp for 3 yr Eruption generalized later; also involved mucosa. Oral & pharyngeal lesions noted	Short course of steroids; later responded to sulphapyridine	Scalp for 3 yr, generalized for 2 yr 10 months	Diabetic; died of unknown causes with active disease
53F	Scalp (cicatricial alopecia). Vaginal & oral bullae also	Systemic steroids; trial of antimalarials	11 yr; disease inactive for past 8 yr	No other disease

* ANA = antinuclear antibody.

We would like to report briefly on nine cases of probable localized pemphigoid. The patients were among 144 patients with bullous and cicatricial pemphigoid seen at the Mayo Clinic between 1968 and 1975. The relevant clinical data are presented in Table 1. None of the cases was included in the series reported by Brunsting & Perry (1957).

COMMENT

Our cases do not show the predominance of males reported in the series by Brunsting & Perry (1957)

TABLE 2. Biopsy and immunofluorescent data on localized and persistent pemphigoid

Age (years) at onset, sex	Biopsy	Immunofluorescence	
		Indirect (serum)	Direct (tissue)
63F	1. SEB* consistent with pemphigoid 2. Ulceration 3. SEB consistent with pemphigoid	1. Neg BMZ†	Not done
29F	1. SEB 2. SEB 3. SEB consistent with pemphigoid	1. Neg BMZ ANA‡ pos 1/20 2. Neg BMZ ANA pos 1/20	1. Linear C3 & fibrin at BMZ
53F	1. SEB 2. SEB 3. SEB with minimal inflammation	1. Neg BMZ	1. Neg 2. Cytoid bodies only (IgM)
79M	1. SEB consistent with erythema multiforme	1. Neg BMZ	1. Linear C3 & fibrin at BMZ
75M	1. SEB 2. Spongiotic vesicles	1. Neg BMZ ANA pos 1/40	1. Linear C3 & fibrin at BMZ; IgA in vessels
57F	1. Ulceration 2. Ulceration 3. SEB consistent with pemphigoid 4. Ulceration	1. Neg BMZ	Not done
77F	1. SEB consistent with pemphigoid	1. Neg BMZ	1. Neg
58M	1. SEB 2. SEB 3. Ulceration	1. Pos BMZ 1/320 2. Pos BMZ 1/160	Not done
53F	1. SEB 2. Ulceration	1. Neg BMZ but done after disease remitted	Not done

* SEB = subepidermal bulla. † BMZ = basement membrane zone. ‡ ANA = antinuclear antibody.

and by MacVicar & Graham (1966). Only three of our patients showed involvement predominantly of the head and neck, while four showed involvement predominantly of the pretibial region, unilateral in only one instance. In two patients the eruption eventually became generalized, and in one patient (58M) it evolved into a sulphapyridine-responsive bullous pemphigoid. This patient was the only one in whom indirect immunofluorescence was positive for circulating antibasement membrane zone antibodies. However, we did not have the opportunity to study two patients (77F, 58M) before their lesions became generalized. One patient (53F) had oral and vaginal lesions typical of cicatricial pemphigoid. Thus lesions remained localized in only six patients, but in one they remained so for 14 years. Only in patients with scalp lesions was scarring obvious. Biopsy findings in all patients were consistent with bullous pemphigoid, but the inflammation was minimal in one. Direct immunofluorescence did not show immunoglobulin deposition in any of the four patients in which it was performed; but a linear deposition of C3 and fibrin was seen at the basement membrane zone in three of the four. We consider this at least highly suggestive of pemphigoid. Interestingly, the lesions in all three borderline-positive patients were localized to relatively sun-exposed areas. The studies of Cram & Fukuyama (1972) showed that the characteristic immunological and histological lesions of pemphigoid can be induced on the normal skin of bullous pemphigoid patients by ultraviolet light. It is therefore reasonable to assume that exposure to the sun may contribute to the pathogenesis of lesions on the head, neck, and forearms. Similarly, vascular disease may have contributed to the pathogenesis of localized pretibial lesions since one of the four patients had varicose veins and another had arteriosclerosis obliterans and intermittent claudication.

In considering our cases with those reported in the literature, it seems plausible that localized pemphigoid occurs in two forms: that type first described by Brunsting & Perry (1957) in which scarring bullous plaques occur on sun-exposed areas, especially on the head and neck, which predominates in males and is resistant to therapy; and the second type, those cases first emphasized by Eberhartinger & Niebauer (1961), in which lesions are more bullous, less cicatricial, less plaque-like, usually occur pretibially in women, and often can be controlled topically. The former seems clinically and probably immunologically related to cicatricial pemphigoid; but little can be said regarding the latter group, as direct immunofluorescence was essentially negative in the two lesions on which it was performed.

ACKNOWLEDGMENT

The immunofluorescent studies were performed in the laboratory of Dr Robert E.Jordon.

REFERENCES

BOURLOND, A., PHILIPPART, J.L. & HOET, J.J. (1970) Pemphigoïde cicatricielle du cuir chevelu. *Archives Belges de Dermatologie et de Syphiligraphie*, **26**, 567.

BRUNSTING, L.A. & PERRY, H.O. (1957) Benign pemphigoid? A report of seven cases with chronic, scarring, herpetiform plaques about the head and neck. *Archives of Dermatology*, **75**, 489.

CRAM, D.L. & FUKUYAMA, K. (1972) Immunohistochemistry of ultraviolet-induced pemphigus and pemphigoid lesions. *Archives of Dermatology*, **106**, 819.

EBERHARTINGER, C. & NIEBAUER, G. (1961) Zur Prognose und Therapie des Pemphigus vulgaris und ähnlicher Erkrankungen. *Hautarzt*, **12**, 503.

FÖLDVÁRI, F. (1958) Pemphigus ou pemphigoïde. *Dermatologica*, **117**, 296.

FRAIN-BELL, W. (1959) Bullous dermatitis and bullous pemphigoid. *British Journal of Dermatology*, **71**, 241.

JONQUIERES, E.D.L. (1965) Circumscribed and atrophying variety of Duhring-Brocq's dermatitis: purely cutaneous variety of the Brunsting and Perry's type of cicatricial pemphigoid. *Revista Argentina de Dermatologia*, **49**, 34.

LEVER, W.F. (1953) Pemphigus. *Medicine (Baltimore)*, **32**, 1.

MACVICAR, D.N. & GRAHAM, J.H. (1966) Localized chronic pemphigoid: a clinicopathologic and histochemical study (abstract). *American Journal of Pathology*, **48**, 52a.

SLEPYAN, A.H., BURKS, J.W. & FOX, J. (1961) Persistent denudation of the scalp in cicatricial pemphigoid: treatment by skin grafting. *Archives of Dermatology*, **84**, 444.

SNEDDON, I.B. (1962) Cicatricial pemphigoid and skin grafts. *Archives of Dermatology*, **86**, 226.

STEWART, W.M., LAURET, P., LECROQ, C. & THOMINE, E. (1971) Pemphigoïde bulleuse et alopécie cicatricielle, pemphigoïde bulleuse et récidive '*in situ*'. *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **78**, 412.

TREPANIER, Y. (1970) Localized bullous dermatitis herpetiformis. *Archives of Dermatology*, **101**, 98.

British Journal of Dermatology (1976) **95**, 535.

Bullous pemphigoid and psoriasis: does subclinical bullous pemphigoid exist?

JOHN R. PERSON AND ROY S. ROGERS III

The Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901, U.S.A.

Accepted for publication 30 March 1976

SUMMARY

Four cases are presented in which probable bullous pemphigoid and probable psoriasis co-existed. One case represents a simple coincidence of the two diseases. In the other three cases, the bullous lesions were transient and seemed to be induced by elements of the Goeckerman regimen. The hypothesis is presented that these three cases represent a subclinical form of bullous pemphigoid induced by the irritant effects of coal tar, ultraviolet light, the reduced barrier function of a pre-existing dermatosis, or a combination of these factors.

Bullous pemphigoid has often been described in association with malignancy (Forman, 1960; Marks, 1961; Ive, 1963; Boyd, 1964; Morandi, Panerai & Bongi, 1964; Skog, 1964; Cormia & Domonkos, 1965; Abadir, 1967; Parsons & Savin, 1968; Rook, 1968; Tye, 1970; Post, Wilhelmi & Jänner, 1973; Saikia, MacKie & McQueen, 1973; Cuni, Grünwald & Rosner, 1974; Zingsheim & Gartmann, 1974) and has sporadically been associated with rheumatoid arthritis (Lillicrap, 1963; Salo & Räsänen, 1972), polymyositis (Peck & Lefkovits, 1966), systemic lupus erythematosus (Jordon *et al.*, 1969), and chronic pancreatitis with prolonged spontaneous hypothermia (Smith, 1966). Recent studies indicate that the association of pemphigoid with malignancy is probably due only to the coincidence of both conditions with increased frequency in elderly patients (Lim, Macdonald & Rook, 1968; Paslin, 1973; Ebner & Spängler, 1974; Stone & Schroeter, 1975). A rare case of drug-induced pemphigoid has been described (Waranicz & Czernielewski, 1974), as has its occurrence after the topical use of 5-fluorouracil for treatment of actinic keratoses (Bart & Bean, 1970). Cram & Fukuyama (1972) found that the characteristic histological and immunohistochemical changes of bullous pemphigoid can be induced in the normal skin of pemphigoid patients by ultraviolet light (UVB).

From August 1973 to August 1975 we saw approximately thirty new patients with bullous pemphigoid at the Mayo Clinic. We wish to report the cases of four patients in whom psoriasis and bullous pemphigoid may have co-existed. In three of the four the lesions were transient and seemed to have been induced by the Goeckerman regimen.

Reprint requests to: Dr J.R.Person, c/o Section of Publications, Mayo Clinic, 200 First Street SW, Rochester, MN 55901, U.S.A.

CASE REPORTS

Case 1. A 53-year-old Iowa mail carrier first presented in December 1973 with a 30-year history of psoriasis vulgaris involving the scalp, elbows, knees and lumbosacral area and a recent bullous eruption which had become quiescent. He was mildly hypertensive and had been taking methyldopa and hydrochlorothiazide (Aldoril) for 4 months. While on the medication, he had noted nausea, dyspepsia, and myalgia. Three weeks before his admission to this clinic he noted the onset of rare, tense bullae in the plaques of psoriasis on his forearms, right wrist, and right knee. He had been giving himself ultraviolet light treatments at home for his psoriasis during this period. A specimen for biopsy was obtained by his home physician and was interpreted at this clinic as 'consistent with bullous pemphigoid'. The patient was free of blisters when he presented for the Goeckerman regimen for his psoriasis.

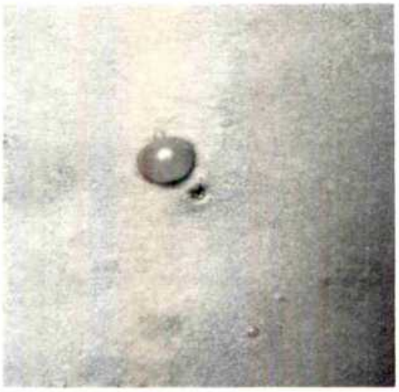


FIGURE 1. Clinical appearance of bulla.

On the 4th day in hospital two bullae developed on the patient's left flank (Fig. 1). Biopsy findings (Fig. 2) were interpreted as consistent with bullous pemphigoid. Direct immunofluorescence studies were not done. Indirect (serum) immunofluorescence showed an antibasement membrane zone titre of 1/5120, and a diagnosis of bullous pemphigoid was considered. The lesions lasted only a few days and

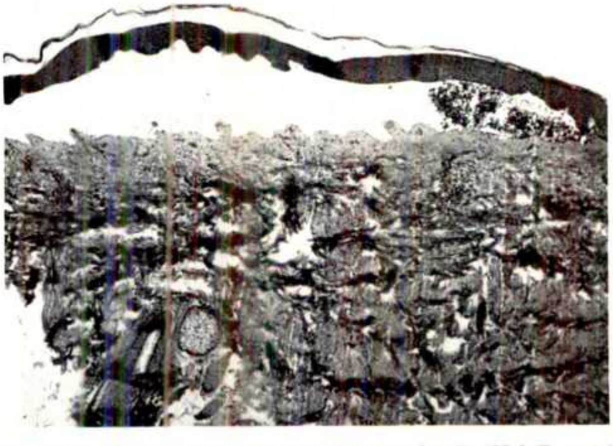


FIGURE 2. Histological evidence in biopsy of bulla (H & E, × 64).

did not return. The patient had not taken Aldoril since 5 days before his hospitalization. Results of a hypertension work-up were negative and the patient's hypertension was adequately controlled with a low salt diet. His psoriasis responded well to the Goeckerman regimen. Communication with the patient in December 1975 indicated that he had not had a recurrence of the bullae, and a repeat indirect immunofluorescence test in November 1975 (23 months after initial admission here) showed an antibasement membrane zone titre of 1/160. Administration of Aldoril was restarted on two occasions after 1973, but was discontinued each time within a few days because of nausea. Otherwise he took no other medication. The psoriasis has been well controlled and he has continued using his ultraviolet light at home during the winter months.

Case 2. A 53-year-old Iowa mail carrier with a history of mild atopy was first seen in May 1975 with an 8-month history of plaques on the elbows and knees that had been diagnosed previously as psoriasis. The eruptions had not cleared in response to topical steroids; indeed 1 month before his admission to the Mayo Clinic, the eruption had become less plaque-like and more erythematous, papular, and migratory. A biopsy specimen from the abdomen was interpreted as probable psoriasis, and treatment was begun with the Goeckerman regimen. Within a day, however, his eruption flared, and several transient bullae were noted on his right shoulder and the scapular region. He had been treated with topical 2% crude coal tar, but ultraviolet light treatments had not been started when the bullae appeared. Two days previously he had started using a diuretic (Dyazide) for mild essential hypertension.

A biopsy was interpreted as showing a subepidermal bulla consistent with dermatitis herpetiformis and direct (tissue) immunofluorescence showed a linear deposition of IgG, C3, and fibrin at the basement membrane zone. Indirect (serum) immunofluorescence was negative on two occasions. The bullae disappeared in a few days and the patient's eruption responded to topical steroids and wet dressings. Patch testing to a standard and a vehicle series showed a 3+ reaction to nickel. Serum IgE was elevated at 2,514 ng/ml. Diagnostic possibilities included psoriasis, contact dermatitis, bullous pemphigoid, or some combination thereof. The patient was considerably improved and was dismissed, only to return 1 week later with a flare-up of his eruption. Biopsy of a lesion on his right arm was interpreted as 'questionable pustular psoriasis', but clinically he was without pustules. Again he responded well to topical steroids and was dismissed after 24 days. Recent communication with the patient indicated that he is still on Dyazide and that the character of his psoriatic eruption has not changed but has remained controllable with topical steroids. Every few weeks he gets an occasional 0·5–1·0 cm, variably pruritic blister, usually on the upper part of the trunk. Recently, blisters first occurred on the forehead during a winter vacation in Arizona.

Case 3. A 70-year-old white man, a retired dry cleaner, presented to this clinic in May 1975 with a 3-week history of a generalized bullous eruption that had begun on his left leg. Oral bullae also were noted. He had a 40-year history of generalized psoriasis with finger and toe arthropathy, occasionally treated with oral corticosteroids. Physical examination disclosed distal interphalangeal and metatarsophalangeal joint deformities and a right inguinal hernia in addition to the dermatological findings. Biopsy of a bulla on the left arm was described as a subepidermal bulla, possibly dermatitis herpetiformis. Direct immunofluorescence of the bulla showed a linear deposition of IgG, C3, and fibrin at the basement membrane zone on two occasions. Indirect (serum) immunofluorescence showed a titre of antibasement membrane antibodies of 1/320 on one occasion, but was negative on two later determinations during treatment. He was found to have mild diabetes mellitus, which was controlled by diet. A roentgenogram of the lumbosacral part of the spinal column showed evidence of early spondylitis and one of the chest showed a granuloma in the right upper lobe. Results of a PPD skin test at 5 and 250 TU (tuberculin units) and an HLA-B27 antigen determination were negative. Treatment with 20 mg of prednisone 4 times daily and use of a diabetic, low salt diet brought a good response. In September treatment with methotrexate by mouth (25 mg/wk) was started. The dosages of steroids were gradually tapered and discontinued in October 1975. By January 1976 the dose of methotrexate had been tapered to 15 mg per week. At the time of this report the patient has only a lumbar plaque of psoriasis and has not had a recurrence of bullae. He is on no medication except methotrexate.

Case 4. A 66-year-old white farmer presented to this clinic in July 1975 with an 18-month history of red-purple and slightly scaly plaques which began on his knees and gradually spread to involve most of his body surface. The lesions were quite pruritic. He had been treated intermittently with numerous systemic corticosteroid preparations without significant benefit. He also gave a history of hand and foot vesicles, clinically most consistent with dyshidrosis. There was no history of atopy in the patient or his family and there was no family history of psoriasis. The patient was on no medications. On examination, plaques were noted on the trunk, arms, legs, face, and scalp, with intertriginous maceration. Mild pitting of the nails was present. The presumptive diagnosis was psoriasis and the patient was hospitalized for the Goeckerman regimen. He was found to have mild diabetes mellitus and mild essential hypertension, both of which responded to dietary therapy. Intravenous pyelography and bolus nephrotomography, however, showed evidence of a left renal mass for which the patient refused surgical treatment or additional investigation. On the 17th hospital day, a blister appeared on his left thigh, biopsy of which showed a subepidermal bulla. Direct immunofluorescence showed linear IgG and C3 at the basement membrane zone and fibrin in the upper dermis. Indirect (serum) immunofluorescence showed 1/320 titre of antibasement membrane zone antibodies. Biopsies of the plaque-like lesions during treatment showed nonspecific dermal inflammation with hyperkeratosis and chronic dermatitis. The eruption responded well to the Goeckerman regimen and he was dismissed on topical therapy alone. A follow-up letter indicated that he has had no further bullae and is on no systemic medications. His psoriasis is well controlled topically.

COMMENT

Of the four cases, only case 3 seemed to represent a simple coincidence of bullous pemphigoid and psoriasis. In the other cases we believe that the reduced barrier function of diseased epidermis, the irritant effects of crude coal tar, the ultraviolet light, or, more likely, a combination of these factors resulted in transient lesions of pemphigoid which otherwise might have remained in a subclinical or dormant state. This presumption is supported by the reports of the induction of bullous pemphigoid by 5-fluorouracil (Bart & Bean, 1970) and ultraviolet light (Cram & Fukuyama, 1972). Case 1 is most convincing because the bullae were noted only in areas of psoriasis and because it is the only case in which we have a relatively long-term follow-up. After 23 months the bullae have not recurred and a moderately high titre of antibasement membrane antibody persists. Case 4 presents convincing histological and immunological evidence that his bullous lesions represent pemphigoid and the transient nature of the lesions suggests external induction. Case 2 is the least convincing, but we believe that the biopsy evidence of probable psoriasis and the direct immunofluorescence typical of bullous pemphigoid allow us to say that the patient is highly suspected of having the two diseases, and that the initially transient nature of the lesions strongly suggests their induction by crude coal tar. Although he has had rare, intermittent bullae since that time, his psoriasiform dermatosis has also been continually active and may be exacerbating his bullous disease. The patient additionally noted an exacerbation after exposure to the sun. Unfortunately, cases 2, 3 and 4 were quite recent and therefore require more prolonged follow-up. Interestingly, two patients (cases 1 and 2) were 53-year-old Iowa mail carriers when their bullous eruption began. The pathogenic significance of snow, rain, heat and gloom of night remains to be investigated.

A significantly positive titre of circulating antibasement membrane zone antibodies generally is considered to be specific for bullous pemphigoid. The data from the laboratory of Jordon, Chorzelski & Beutner (1970), who also reported the findings of Peck and associates, did not show any false positives in 2909 controls. Thivolet *et al.* (1968) and, more recently, Rodriguez and Bystryn (1975) have shown a 3% incidence of false-positive controls among normals and miscellaneous dermatoses. It would be of considerable interest to attempt photo-induction with or without crude coal tar in any truly positive controls because some of these may represent subclinical bullous pemphigoid.

ACKNOWLEDGMENT

Immunofluorescent studies were performed in the laboratory of Dr Robert E.Jordon.

REFERENCES

ABADIR, R. (1967) Pemphigoid, bronchial neoplasm and radiotherapy. *Proceedings of the Royal Society of Medicine*, **60**, 1271.

BART, B.J. & BEAN, S.F. (1970) Bullous pemphigoid following the topical use of fluorouracil. *Archives of Dermatology*, **102**, 457.

BOYD, R.V. (1964) Pemphigoid and carcinoma of the pancreas. *British Medical Journal*, **i**, 1092.

CORMIA, F.E. & DOMONKOS, A.N. (1965) Cutaneous reactions to internal malignancy. *Medical Clinics of North America*, **49**, 655.

CRAM, D.L. & FUKUYAMA, K. (1972) Immunohistochemistry of ultraviolet-induced pemphigus and pemphigoid lesions. *Archives of Dermatology*, **106**, 819.

CUNI, L.J., GRÜNWALD, H. & ROSNER, F. (1974) Bullous pemphigoid in chronic lymphocytic leukemia with the demonstration of antibasement membrane antibodies. *American Journal of Medicine*, **57**, 987.

EBNER, H. & SPÄNGLER, E. (1974) Untersuchungen über die Beziehung von bullösem Pemphigoid und malignen Allgemeinerkrankungen. *Wiener Klinische Wochenschrift*, **86**, 449.

FORMAN, L. (1960) Pemphigoid occurring with carcinoma of the rectum. *Proceedings of the Royal Society of Medicine*, **53**, 563.

IVE, F.A. (1963) Metastatic carcinoma of cervix with acanthosis nigricans, bullous pemphigoid and hypertrophic pulmonary osteoarthropathy. *Proceedings of the Royal Society of Medicine*, **56**, 910.

JORDON, R.E., CHORZELSKI, T.P. & BEUTNER, E.H. (1970) Clinical significance of autoantibodies in bullous pemphigoid. In: *Autosensitization in Pemphigus and Bullous Pemphigoid* (Ed. by E.H.Beutner, T.P.Chorzelski and R.E.Jordon), p. 94. Charles C.Thomas, Springfield, Illinois.

JORDON, R.E., MULLER, S.A., HALE, W.L. & BEUTNER, E.H. (1969) Bullous pemphigoid associated with systemic lupus erythematosus. *Archives of Dermatology*, **99**, 17.

LILLICRAP, D.A. (1963) Rheumatoid arthritis and pemphigoid. *Proceedings of the Royal Society of Medicine*, **56**, 921.

LIM, C.C., MACDONALD, R.H. & ROOK, A.J. (1968) Pemphigoid eruptions in the elderly. *Transactions of the St John's Hospital Dermatological Society*, **54**, 148.

MARKS, J.M. (1961) Pemphigoid with malignant melanoma. *Proceedings of the Royal Society of Medicine*, **54**, 225.

MORANDI, G.A., PANERAI, A. & BONGI, G. (1964) Le sindromi paraneoplastiche. III. Contributo clinico alla conoscenza del pemfigoide paraneoplastico. *Rivista Critica di Clinica Medica*, **64**, 498.

PARSONS, R.L. & SAVIN, J.A. (1968) Pemphigoid and malignancy. *British Journal of Cancer*, **22**, 669.

PASLIN, D.A. (1973) Bullous pemphigoid and hypernephroma: a critical review of the association of bullous pemphigoid and malignancy. *Cutis*, **12**, 554.

PECK, S.M. & LEFKOVITS, A.M. (1966) Bullous pemphigoid with polymyositis and co-existing contact dermatitis. *Archives of Dermatology*, **94**, 672.

POST, B., WILHELMI, F. & JÄNNER, M. (1973) Bullöses Pemphigoid als cutanes paraneoplastisches Syndrom bei einem Gallenblasencarcinom. *Hautarzt*, **24**, 193.

RODRIGUEZ, J. & BYSTRYN, J.-C. (1975) The reliability of the indirect immunofluorescent test for pemphigus and bullous pemphigoid (abstract). *Clinical Research*, **23**, 454.

ROOK, A.J. (1968) A pemphigoid eruption associated with carcinoma of the bronchus. *Transactions of the St John's Hospital Dermatological Society*, **54**, 152.

SAIKIA, N.K., MACKIE, R.M. & MCQUEEN, A. (1973) A case of bullous pemphigoid and figurate erythema in association with metastatic spread of carcinoma. *British Journal of Dermatology*, **88**, 331.

SALO, O.P. & RÄSÄNEN, J.A. (1972) Pemphigoid and rheumatoid arthritis: a clinical and immunological study. *Annals of Clinical Research*, **4**, 173.

SKOG, E. (1964) Cutaneous manifestations associated with internal malignant tumours with particular reference to vesicular and bullous lesions. *Acta dermato-venereologica (Stockholm)*, **44**, 114.

SMITH, G.M. (1966) Prolonged spontaneous hypothermia: report of a case associated with chronic pancreatitis and a pemphigoid eruption. *Annals of Internal Medicine*, **65**, 316.

STONE, S.P. & SCHROETER, A.L. (1975) Bullous pemphigoid and associated malignant neoplasms. *Archives of Dermatology*, **111**, 991.

THIVOLET, J., BEYVIN, A.-J., LEUNG, T.K. & ANDRÉ, M. (1968) Maladie de Duhring-Brocq et anticorps anti-membrane-basale. *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **75**, 639.

TYE, M.J. (1970) Bullous pemphigoid and Kaposi's sarcoma. *Archives of Dermatology*, **101**, 690.

WARANICZ, A. & CZERNIELEWSKI, A. (1974) Pemfigoid sproowokowany nowoskabina [Pemphigoid induced by Novoscabin]. *Przeglad Dermatologiczny*, **41**, 639.

ZINGSHEIM, M. & GARTMANN, H. (1974) Bullöses Pemphigoid und figurierte Erytheme als paraneoplastische Hautreaktion bei einem Bronchialkarzinom. *Zeitschrift fur Hautkrankheiten*, **49**, 215.

British Journal of Dermatology (1976) **95**, 541.

Bullous dermatosis of chronic renal failure

KALMAN KECZKES AND MALCOLM FARR

Departments of Dermatology and Nephrology, Hull Royal Infirmary, Hull, North Humberside

Accepted for publication 14 April 1976

SUMMARY

Four of five patients with chronic renal failure developed tense bullae on the backs of their hands and fingers. In one of these patients the bullae were more widespread. In the fifth patient the lesions were limited to the fingers. The clinical picture resembled that of porphyria cutanea tarda, but porphyrin studies were consistently within normal limits. Histological studies showed subepidermal pressure bullae in each patient. In one of the patients immunohistology showed greenish fluorescence at the bases of the dermal papillae.

In 1975 Gilchrest, Rowe & Mihn reported a bullous dermatosis of the light exposed areas, primarily on the backs of the hands, in five patients on maintenance haemodialysis. Porphyrin studies and photo-testing were normal in all of them. In the same year Korting (1975) reported two female patients, also on maintenance haemodialysis, who developed bullae and erosions on the backs of their hands. In one of them raised amounts of uroporphyrin I and III were present in the urine.

We here report five more patients with chronic renal failure who developed during the unusually hot summer, in England, in 1975, bullae on sun exposed areas. These lesions resembled the skin lesions of porphyria cutanea tarda although porphyrin studies were normal.

CASE REPORTS

Case 1

C.G., a 50-year-old married woman with analgesic nephropathy, was admitted in September 1974 with chronic renal failure. She underwent peritoneal dialysis in October 1974 with generalized improvement. Her renal function, however, deteriorated by the spring of 1975. In May 1975 the creatinine clearance was 2 ml/min. In June 1975 tense bullae were noted on the backs of the fingers of both hands. The lesions recurred and subsided after bursting. She was first seen at the Department of Dermatology in July 1975. On examination large tense bullae and some denuded areas and crusts were present on the backs of the fingers and hands. There were no milia present. The rest of the skin was not involved. Her condition rapidly deteriorated and she died at the beginning of August 1975. Haemodialysis was not instituted.

When the bullae appeared she was taking the following medication: frusemide, digoxin, allopurinol, sodium bicarbonate, aluminium hydroxide, folic acid, iron, phenytoin, clonidine and calcium carbonate.

The chemical screening test for erythropoietic protoporphyria was negative. Urinary porphyrins were within normal limits. Skin biopsy was not performed.

Case 2

P.W., a 25-year-old man with end stage renal failure, had been on maintenance haemodialysis for 18 months. In mid-July 1975 he developed symptomless blisters on the backs of his fingers and hands. On examination there were tense bullae and some dried crusted areas on the backs of his fingers and knuckles. There were no milia present.

When the bullae appeared he was taking the following medication: frusemide, methyldopa, aluminium hydroxide and polystyrene sulphonate.

Histology revealed a subepidermal bulla (Fig. 1). Chemical screening test for erythropoietic protoporphyria was negative. Urinary and faecal porphyrins were within normal limits.

Although topical corticosteroids and chlorpheniramine maleate were instituted, bullae re-appeared until October 1975.

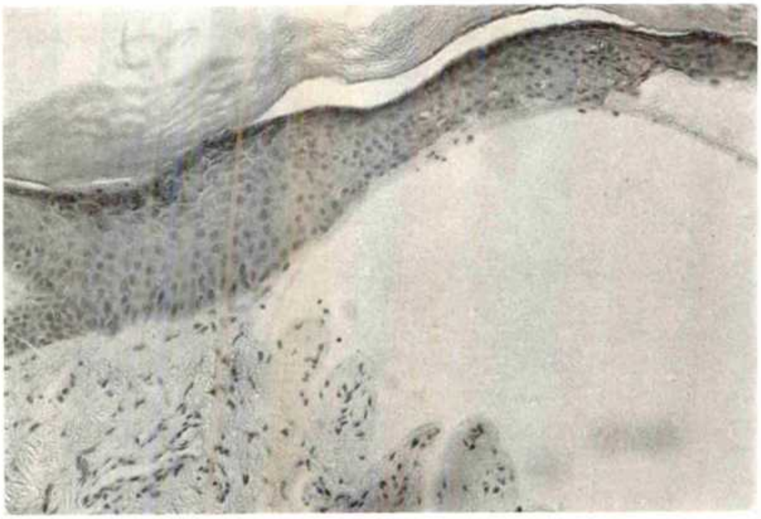


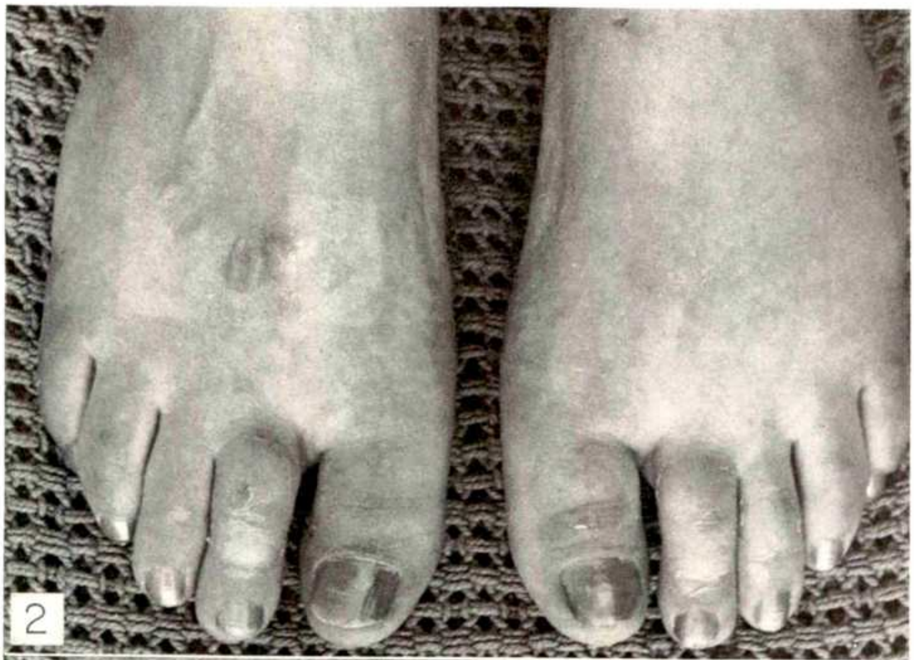
FIGURE 1. Subepidermal pressure bulla (H & E, × 100).

Case 3

R.W., a 22-year-old man with proliferative glomerulonephritis, developed the nephrotic syndrome, hypertension and progressive renal failure. He gave a history of blisters appearing on the backs of the hands and forearms 4 years previously following exposure to sunlight. There was no family history of light sensitivity. In mid-July 1975, when the creatinine clearance was approximately 5 ml/min, he developed blisters on the backs of his hands and fingers, but not elsewhere. On examination there were tense bullae, some haemorrhagic, and a few crusted areas on the backs of the fingers and hands, but no milia. In September 1975 he underwent bilateral nephrectomy. Since then he has been on maintenance haemodialysis. Bullae did not recur after October 1975.

Medication included: frusemide, calcium polystyrene sulphonate, calcium carbonate, aluminium hydroxide and methyldopa.

Histology revealed a subepidermal bulla. Chemical screening test for erythropoietic protoporphyria was negative. Urinary and faecal porphyrins were within normal limits.



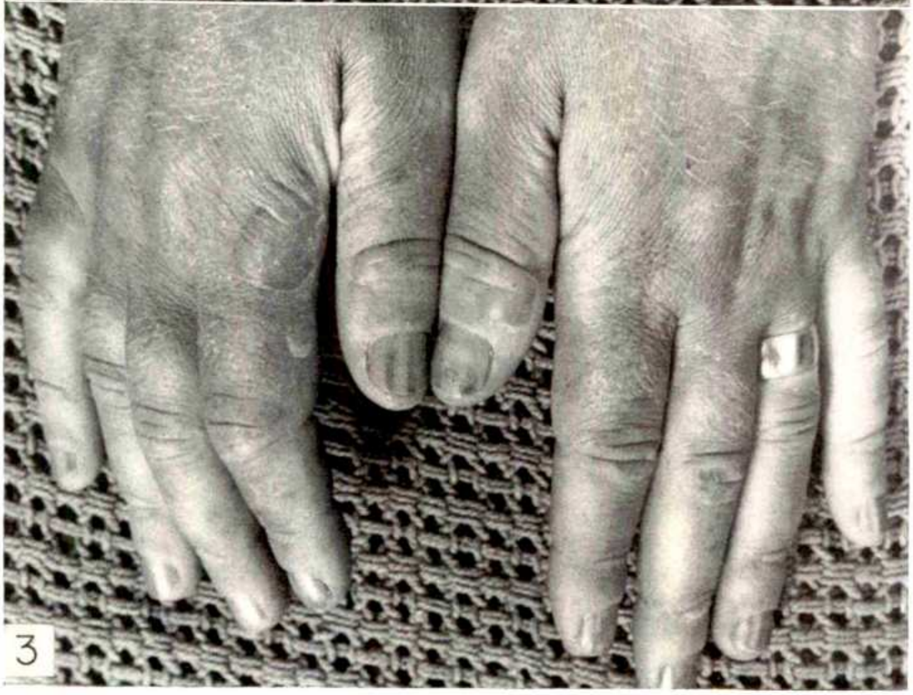


FIGURE 2. Resolving bullae on backs of toes and feet.

FIGURE 3. Tense bullae on the backs of the fingers and thumbs.

Case 4

L.T., a 29-year-old married woman with probable chronic pyelonephritis, had been on maintenance haemodialysis for 4 years. In May 1975 a renal transplantation was performed. The graft functioned well after initial haemodialysis. In mid-July 1975, when the creatinine clearance was 34 ml/min, she developed blisters on the backs of her toes (Fig. 2). Later she developed blisters on the backs of her fingers and hands (Fig. 3). Similar lesions also appeared on her trunk, nose, right ear and lip. There was no history of previous skin disease or photo-sensitivity. On examination there were tense bullae on the backs of the fingers and toes with some crusted areas on the backs of the hands, but no milia.

When the bullae appeared medication included: frusemide, azathioprine, prednisolone and sodium bicarbonate.

Histology revealed a subepidermal bulla. Direct immunofluorescent techniques revealed greenish fluorescence at the bases of dermal papillae on sections stained for IgG. Indirect immunofluorescent preparations using the patient's serum and normal human skin were negative.

Chemical screening test for erythropoietic protoporphyria was negative. Urinary and faecal porphyrins were with normal limits.

With chlorpheniramine maleate orally and topical corticosteroids the bullae disappeared and the skin recovered fully by October 1975.

Case 5

R.D., a 55-year-old housewife with adult polycystic kidney disease and a creatinine clearance of 13 ml/min, developed a few small blisters on her fingers. These soon resolved. Her medication included: frusemide, methyldopa and aluminium hydroxide.

Histology was not performed. Chemical screening test for erythropoietic protoporphyria was negative. Urinary and faecal prophyrins were within normal limits. The patients were genetically unrelated and came from various parts of the east of England.

Other investigations revealed low serum IgA levels in four, low serum IgG levels in five and elevated serum IgM levels in three. Serum complement (C3) levels varied from low to high. The anti-nuclear factor was negative in all five patients and the Australia antigen test was negative in four of the five patients tested. Viral serology performed in four of the five patients was non-contributory. Tissue typing revealed HL-A 2 to be present in three of the four patients tested. The donor of the graft was also HL-A 2.

DISCUSSION

Gilchrest *et al.* (1975) described five patients with chronic renal failure, on intermittent haemodialysis, who developed bullae on the backs of the hands. The distribution and appearance of the lesions were strongly suggestive of porphyria cutanea tarda. Porphyrin studies, however, revealed no abnormality in any of them. Several features suggested light sensitivity but photo-testing was negative in all patients. In each patient the bullae subsided after a few months when the weather became milder. Histological examination showed subepidermal bullae. Immunofluorescent studies were negative in one of the patients, but in three patients IgG was deposited around superficial dermal venules and in two, IgA deposits were also demonstrated. Stains for complement (C3) were negative in all patients.

Korting (1975) reported two female patients with chronic renal failure on intermittent haemodialysis who developed bullae and erosions on the backs of their hands, strongly suggestive of porphyria cutanea tarda. In one patient porphyrin studies were repeatedly normal, in the other one the amounts of uroporphyrin I and III were raised in the urine.

Four of our five patients developed skin lesions on the backs of their fingers and hands. One of them had bullae on the backs of the toes and feet, and on the trunk, ear, nose and lip. In another the lesions were limited to the fingers. These tense bullae, which soon ruptured and left behind crusted areas, were strongly suggestive of porphyria cutanea tarda, although no patients developed milia on the skin and porphyrin studies were within normal limits in all patients.

Standard light microscopic examination of histological preparations stained with haematoxylin and eosin showed subepidermal bullae in three of them. Immunofluorescent studies were performed in only one of the patients. In this patient the direct immunofluorescent technique revealed greenish fluorescence at the bases of dermal papillae on sections stained for IgG. Indirect immunofluorescent preparations using the patient's serum and normal human skin were negative.

The histological features in light microscopical sections could suggest bullous pemphigoid. Bullous pemphigoid was reported by Bean, Jordan & Windhorst (1970) in an 11-year-old boy who had persistent proteinuria and in whom renal biopsy showed mesangial thickening. Similarly Esterley *et al.* (1973) reported a 12-year-old girl with bullous pemphigoid and membranous glomerulonephropathy. The distribution of the lesions and the short course of the disease in our patients, however, would not suggest that diagnosis.

As early as 1888 Osler recognized the association of erythema multiforme and nephritis, which was confirmed later by several authors (Comaish & Kerr, 1961; Bloom & Lovel, 1964; Bluefarb & Szanto, 1965). The course of the disease and histological features of our patients, however, would not be consistent with that diagnosis.

The underlying renal disease was probably different in each patient. The creatinine clearance of the five patients ranged from 2 to 34 ml/min. The most widespread lesions developed in the transplanted patient with the best renal function. Whilst all the reported patients (Gilchrest *et al.*, 1975; Korting, 1975) were on maintenance haemodialysis, only one of our patients was undergoing this form of treatment. The only one form of medication common to each of them was frusemide. The dose, however, varied. C.G. with a creatinine clearance of 2 ml/min was taking 2000 mg of frusemide a day. L.T. with the highest creatinine clearance (34 ml/min) was taking only 40 mg daily.

TABLE 1. Hours of sunshine per month as reported by the Meteorological Office for the Hull area

Year	June	July	August	September
1975	256·6	211·5	246·6	143·2
Mean figures for 1941–1970	189·3	170·6	158·0	128·7

Neither the clinical course nor the laboratory studies suggested a viral aetiology in our patients. The summer of 1975 was exceptional, with hours of sunshine well above the average (Table 1). As virtually all the skin lesions of the reported cases, including our own, occurred on sun exposed areas, light sensitivity with or without frusemide appears to be an important aetiological factor.

In conclusion we believe bullous dermatosis of chronic renal failure is a recently recognized association. At present insufficient data are available to determine the exact course. It would appear, however, that some factor(s) other than uraemia itself must be responsible for it.

ACKNOWLEDGMENTS

The authors wish to acknowledge the generous secretarial help of Miss M.Sephton and Miss S. Botterill in the preparation of this paper.

REFERENCES

BEAN, S.F., JORDAN, R.E. & WINDHORST, D.B. (1970) Bullous pemphigoid in an 11-year-old boy. *Archives of Dermatology*, **102**, 205.

BLOOM, A. & LOVEL, T.W.I. (1964) Erythema multiforme with renal and myocardial injury. *Proceedings of the Royal Society of Medicine*, **57**, 175.

BLUEFARB, S.M. & SZANTO, P. (1965) Erythema multiforme associated with acute renal tubular necrosis. *Archives of Dermatology*, **92**, 367.

COMAISH, J.S. & KERR, D.N.S. (1961) Erythema multiforme and nephritis. *British Medical Journal*, **ii**, 84.

ESTERLY, N.B., GOTOFF, S.P., LOLEKHA, S., MOORE, E.S., SMITH, R.D., MEDENICA, M. & FUREY, N.L. (1973) Bullous pemphigoid and membranous glomerulonephropathy. *Journal of Paediatrics*, **83**, 466.

GILCHREST, B., ROWE, J.W. & MIHN, M.C. (1975) Bullous dermatosis of haemodialysis. *Annals of Internal Medicine*, **83**, 480.

KORTING, G.W. (1975) Über Porphyria-cutanea-tarda-artige Hautveränderumgen bei Langzeithämodialysepatienten. *Dermatologica*, **150**, 58.

British Journal of Dermatology (1976) **95**, 547.

Case Report

Caustic ulcers caused by cement

MATTI HANNUKSELA, RAIMO SUHONEN AND JAAKKO KARVONEN

Departments of Dermatology, University Central Hospital, Helsinki, and
University Central Hospital, Oulu, Finland

Accepted for publication 9 April 1976

SUMMARY

Seven similar cases with deep ulceration probably due to wet cement are presented. Within 12 hours after making a concrete floor in a kneeling position, curved ulcers developed on both sides of both patellae. The exposure time varied from 2 to 6 h. One patient got wet concrete into her boots and, consequently, ulcers developed on her feet. The high alkalinity of calcium hydroxide in wet cement was the only apparent causative agent in every case.

Wet concrete is very alkaline due to calcium hydroxide. Its pH is about 12·4–12·5. In fact, calcium lye is the primary cause of toxic hand eczema frequently seen in cement workers. In 1½ years we have seen seven patients with a rare complication probably due to the alkalinity of wet concrete, namely caustic ulcers on the lower extremities.

CASE REPORTS

Table 1 summarizes the most important data from our seven patients. In six cases (nos 2–7), the lesions were nearly identical: curved ulcers on both sides of both patellae (Figs 2 and 3). All the patients had been making a cement floor by levelling wet concrete in a kneeling position for 2–6 h. Patients 6 and 7 were making the same floor. Patient no. 1 got wet concrete into her boots and deep ulcers consequently developed on her feet. The deeper the ulcers were the slower the healing. With the exception of patient no. 3, the subjects were not aware of the irritant properties of fresh wet concrete. Two patients were construction workers (nos 3 and 5) but the remaining five cases handled cement casually. Additives were not used in any of the consignments, all of which were of Finnish cement made by at least 4 manufacturers. Routine epicutaneous tests were performed in four cases with negative results. The patients were otherwise in good health.

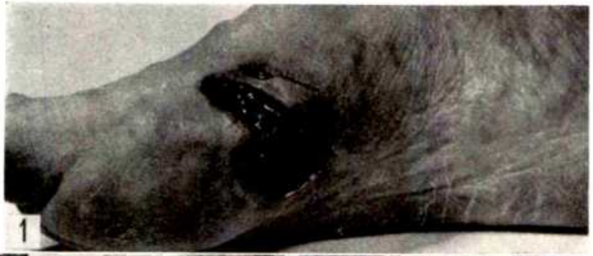
DISCUSSION

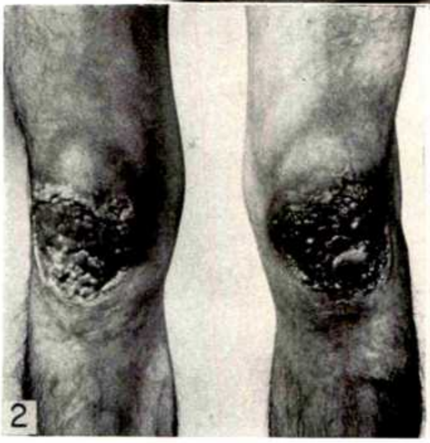
Features common to these seven cases were: 2–6 h exposure to calcium lye, insufficient protection allowing the clothes to get wet, and pressure on the exposed areas. Smarting was the only sensation during the work. However, immediately after stopping work the patients noticed that the exposed skin was already red. Deep ulcers developed within 12 h.

Requests for reprints: Dr M. Hannuksela, Department of Dermatology, University Central Hospital, FIN-90220 Oulu 22, Finland.

TABLE 1. Clinical data of seven patients with caustic ulcers probably due to wet cement

Case no.	Age	Sex	Occupation	Work which caused the ulcers	Protection	Exposure time (h)	Symptoms during the work	Development of ulcers, healing, other remarks
1	34	F	Housewife	Laying a foundation. Wet concrete got into her boots	Nil	6	Nil	In the evening skin on the feet was irritated and blotchy red. The next morning necrosis on both sides of the left foot (Fig. 1) and on the III–IV toes of the right foot. Healing was complete in 14 weeks
2	37	M	Electrician	Making a concrete floor in a kneeling position	Nil	5	Nil	Curved, reddish-black necrotic areas on both sides of both patellae the same evening. In 2 weeks he was fully recovered
3	29	M	Construction worker	As above	Two pairs of trousers, rubber cloth, piece of plank	6	Nil	Wide dull-red necrotic areas developed around both patellae within 12 h (Fig. 2). Recovery took 13 weeks
4	63	M	Master mechanic	As above	Nil	2	Nil	The same evening the skin on the knees was dark red, and during the night necrotic ulcers of the same form as in cases 2 and 3 developed. After 10 weeks only scars could be seen (Fig. 3)
5	32	M	Construction worker	As above	Polystyrene sheet (2″)	4	Smarting	The skin on both knees turned red the same night, and in the morning curved necrosis was found around both patellae. Healed within 7 weeks
6	36	M	Bricklayer	As above	As above	4	Smarting	Lesions identical to cases 2–5 developed during the night and healed in 2 weeks
7	33	M	Bricklayer	As above	As above	4	Smarting	As above





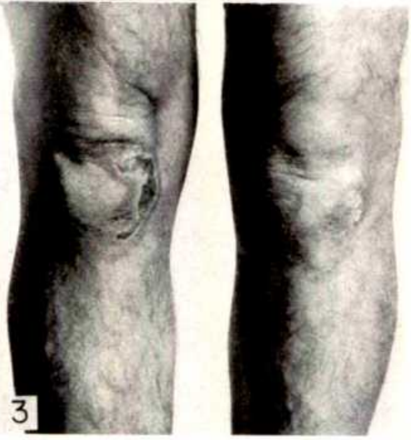


FIGURE 1. Deep ulcer on the foot of patient 1 about 7 weeks after the exposure to wet concrete.

FIGURE 2. Deep, already granulating, necrotic ulcers on the patellar regions of patient 2 at 5 weeks after the exposure to wet concrete.

FIGURE 3. Scars on the patellar regions of patient 4 at 10 weeks.

The composition of Finnish cement has not been changed for decades. Additives were not used in any instance. Thus, it seems probable that calcium hydroxide was the only cause of these caustic lesions representing 2nd–3rd degree burns.

Curiously enough, we have found only one similar case from the literature. Inquiries made among Finnish dermatologists and manufacturers of concrete have been fruitless. Thus, the actual reason for this accumulation of seven fairly similar cases remains obscure. In 1962, Rowe presented one patient with ulceration on both his legs after 2½ h kneeling in wet concrete. This case report was the only one cited by Pittelkow (1975) in Zenz's Occupational Medicine.

ADDENDUM

Recently Vickers & Edwards (*Contact Dermatitis*, 1976, **2**, 73) reported four cases of burns after contact with premixed concrete. The mechanism of these burns was apparently the same as in our seven cases. The main problem whether or not these accumulations of similar cases are due to mere chance remains unresolved.

REFERENCES

ROWE, R.J. (1962) Unusual reaction to cement. *The Wisconsin Medical Journal*, **61**, 395.

PITTELKOW, R. (1975) Occupational dermatoses. Cement dermatitis. In: *Occupational Medicine. Principles and Practical Applications* (Ed. by C.Zenz), pp. 222–223. Year book Medical Publishers, Inc. Chicago.

British Journal of Dermatology (1976) **95**, 551.

Metastatic Crohn's disease

D.I.McCALLUM

Raigmore Hospital, Inverness

W.M.GRAY

City Hospital, Nottingham

Accepted for publication 26 March 1976

SUMMARY

Metastatic Crohn's disease is a term used to describe a granulomatous reaction occurring in flexures, and separated from the affected areas of the gastro-intestinal tract by normal skin. Previous reports refer to such lesions affecting the groins, the male genitalia, a flexure on the anterior abdominal wall of an obese patient, and submammary regions. A further case, in which the retro-auricular areas are involved, is presented.

Dermatological manifestations of Crohn's disease can be divided into two broad groups, (i) those in which histopathological examination of biopsy tissue demonstrates certain characteristic, although not diagnostic, features of the disease, and (ii) those in which there are no such pathological changes. The former group includes peri-anal lesions, extension of the disease process onto the anterior abdominal wall, usually following surgical intervention, granulomatous oral lesions, and what has been referred to as metastatic Crohn's disease, affecting the groins, the male genitalia, an abdominal crease in an obese patient, and submammary regions.

The second group includes a wide variety of dermatoses including erythema nodosum, erythema multiforme (Brenner & Delaney, 1972), pyoderma grangrenosum (Stathers, Abbott & McGuiness, 1967), the effects of malnutrition, palmar erythema, rosacea, epidermolysis bullosa acquisita (Pegum & Wright, 1973), and cutaneous polyarteritis nodosa.

The relationship of the former group with Crohn's disease would seem to be established by the combination of clinical and pathological changes; that of the latter group can, as yet, only be inferred by the clinical association of the disease processes, and by a tendency for the skin lesions to wax and wane in conjunction with those of the gastro-intestinal tract.

The histological features which characterize Crohn's disease of the peri-anal skin have been described by Gray, Lockhart-Mummery & Morson (1965) as collections of epithelioid cells and giant cells of the Langhans type without any central caseation—the sarcoid reaction—infiltrated with and surrounded by variable numbers of lymphocytes and plasma cells. The granulomata may be of variable size, some having less than a dozen epithelioid cells and one or two giant cells, and others containing many dozens of epithelioid cells. In some instances they may be sparse, whilst in other areas the skin may be riddled with lesions, which are found immediately beneath the epidermis, in the dermis, in subcutaneous fat and in muscle, or in the granulomatous tissue lining fistulous tracks, ulcers or abscess cavities.

The clinical features of peri-anal and anterior abdominal Crohn's disease have been described in a previous communication (McCallum & Kinmont, 1968), and subsequently these and other dermatological manifestations of Crohn's disease and of ulcerative colitis have been detailed (Verbov & Stansfield, 1972; Verbov, 1973).

Mouth ulcers complicating Crohn's disease were referred to as aphthous, until Dudeney and Todd (1969) presented a case of 'Crohn's disease of the mouth' at the section of proctology of the Royal Society of Medicine. Since then there have been at least nine further reports of granulomatous changes in the mouth of patients suffering from Crohn's disease. These reports have been reviewed by Taylor & Smith (1975) who themselves described a patient with oral manifestations of Crohn's disease, without demonstrable gastro-intestinal lesions. Croft & Wilkinson (1972) reviewed retrospectively 332 cases of Crohn's disease, and found that 6·1% suffered from oral ulceration at some time in their illness. In one instance the pharynx and larynx were involved.

Metastatic Crohn's disease

Parks, Morson & Pegum (1965) presented a case at the dermatological section of the Royal Society of Medicine, under the title of 'Crohn's disease with cutaneous involvement'. The patient was a 70-year-old female who had developed diarrhoea in 1959, and at the time of presentation, suffered from peri-anal ulceration which extended to the groins. She also had extensive ulceration under the right breast. On biopsy, the submammary lesion was reported as showing chronic inflammation with a florid sarcoid reaction in the dermis. In the subsequent discussion, Morson stated that the particular interest in the case was the presence of the submammary lesion, remote from the gastro-intestinal tract and separated from it by normal skin, and this he referred to as Metastatic Crohn's. Mountain (1970) described this and two further cases of metastatic Crohn's disease involving the penis, and a crease on the anterior abdominal wall of an obese female. In each case the histopathological picture supported a diagnosis of Crohn's disease.

CASE REPORT

As such cases of 'metastatic' Crohn's disease would appear to be uncommon, the following report may be of interest.

In March 1960, a male patient, aged 38 years, was admitted to the City Hospital, Nottingham, for treatment of acute cellulitis of the right thigh and leg. Multiple incisions from the buttock to the popliteal region were required to remove extensive necrotic material, and to release large quantities of thin foul smelling pus which, on culture, grew a mixture of non-haemolytic streptococci, *E. coli* and *Ps aeruginosa*. By May 1960, after a stormy illness, he had responded satisfactorily to antibiotic and supportive therapy, but he still had discharging sinuses on the posterior aspect of the thigh; these have persisted to the present time. In November 1962, a sinus track was excised from the right thigh and sent for histopathological examination. It was reported initially as showing non-specific inflammatory changes, but when the sections were reviewed in 1966, by which time the clinical condition had many of the features of Crohn's disease, the pathologist reported that the histological appearances supported the clinical diagnosis. In May 1963, a small fistula appeared to the right of the anal canal, and within 2 months two further peri-anal fistulae had developed. An extensive sinus then developed on the posterior aspect of the left thigh, and in October 1968, a sinogram showed a broad track extending upwards to the peri-anal region, and from this a smaller sinus tracked upwards to the lower rectum.

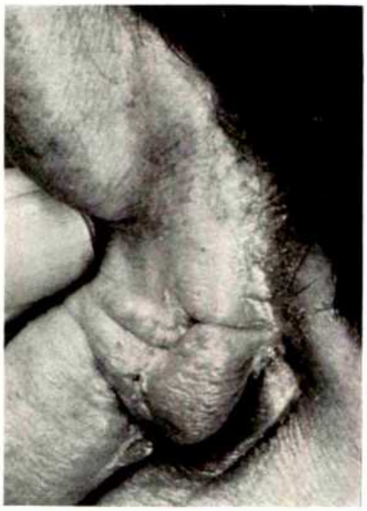


FIGURE 1. Post auricular granulomata with sinuses (L. side).

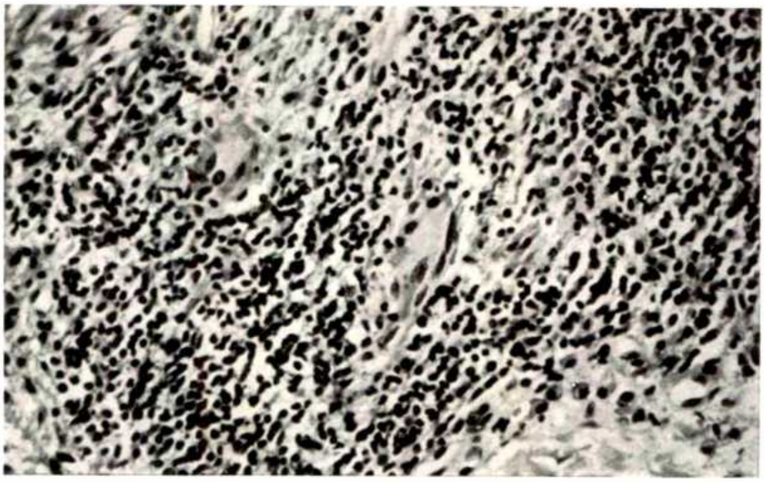


FIGURE 2. Dermal infiltrate with lymphocytes, histiocytes and giant cells.

At this stage a diagnosis of Crohn's disease of the rectum and anus was established by sigmoidoscopy and biopsy.

In July 1970, granulomatous lesions were apparent in the right retro-auricular area, and 2 years later similar lesions appeared on the left side. The patient had a seborrhoeic type of skin, but there was no antecedent history of flexural eczema or of fissuring in the retro-auricular folds. The granulomata started in the depth of the folds; they were raised, oedematous, of darkish colour, and they had sinuses which discharged a foul smelling exudate. The condition was painless, as were the chronic sinuses on the thighs. There was no associated lymphadenopathy. A biopsy from the right side was reported as showing changes similar to those seen in anal lesions of Crohn's disease. Azathioprine

(50 mg twice daily) was given for 2 weeks and then discontinued as the patient had abdominal pain which he attributed to the drug. The skin lesions in the peri-anal and retro-auricular areas improved at this time, but this may well have been a spontaneous remission, and not associated with the azathioprine. By July 1975, the left retro-auricular condition had settled, and that on the right side was reduced to a small oedematous plaque.

COMMENT

The aetiology of Crohn's disease is still completely obscure as can be judged by the fact that Kyle (1972) considered in detail as possible causes of the disease process lymphatic stasis, trauma, ingestion of foreign material, diverticula, psychological factors and an abnormal immune reaction. Although the histology is described as sarcoid-like there is, as yet, little evidence to support the view that Crohn's disease is a manifestation of sarcoidosis. In Crohn's disease there are protean clinical manifestations affecting the gut, the skin and the mouth. Those in which there is a characteristic histology and also those demonstrating reaction patterns such as erythema nodosum, erythema multiforme, pyoderma gangrenosum and polyarteritis nodosa would suggest that this is a systemic condition, and that the changes could well be accounted for, in part at least, by an abnormal immune reaction.

Mountain (1970) noted that ulceration in metastatic Crohn's disease occurred in sites where there was close apposition of two skin surfaces, and where the surfaces tended to be rather moist. In the present case the depths of the retro-auricular folds were involved, but although the patient had a seborrhoeic skin, there was no history of eczema, intertrigo or fissuring. In peri-anal and metastatic Crohn's disease, there is usually no evidence of associated adenopathy; this is in contrast to gastro-intestinal Crohn's disease in which the lymphatics are markedly involved.

REFERENCES

BRENNER, S.M. & DELANEY, H.M. (1972) Erythema multiforme and Crohn's disease of the large intestine. *Gastroenterology*, **62**, 479.

CROFT, C.B. & WILKINSON, A.R. (1972) Ulceration of the mouth, pharynx and larynx. *British Journal of Surgery*, **59**, 249.

DUDENEY, T.P. & TODD, I.P. (1969) Crohn's disease of the mouth. *Proceedings of the Royal Society of Medicine*, **62**, 1237.

GRAY, B.K., LOCKHART-MUMMERY, H.E. & MORSON, B.C. (1965) Crohn's disease of the anal region. *Gut*, **6**, 515.

KYLE, J. (1972) *Crohn's Disease*, 1st edn, p. 26. Heinemann, London.

MCCALLUM, D.I. & KINMONT, P.D.C. (1968) Dermatological manifestations of Crohn's disease. *British Journal of Dermatology*, **80**, 1.

MOUNTAIN, J.C. (1970) Cutaneous ulceration in Crohn's disease. *Gut*, **11**, 18.

PARKS, A.G., MORSON, B.C. & PEGUM, J.S. (1965) Crohn's disease with cutaneous involvement. *Proceedings of the Royal Society of Medicine*, **58**, 241.

PEGUM, J.S. & WRIGHT, J.T. (1973) Epidermolysis acquisita and Crohn's disease. *Proceedings of the Royal Society of Medicine*, **66**, 234.

STATHERS, G.M., ABBOTT, L.G. & MCGUINNESS, A.E. (1967) Pyoderma gangrenosum in Crohn's disease. *Archives of Dermatology*, **95**, 375.

TAYLOR, V.E. & SMITH, C.J. (1975) Oral manifestations of Crohn's disease without demonstrable gastro-intestinal lesions. *Oral Surgery*, **39**, 58.

VERBOV, J. (1973) The skin in patients with Crohn's disease and ulcerative colitis. *Transactions of the St John's Hospital Dermatological Society*, **59**, 30.

VERBOV, J. & STANSFIELD, A.G. (1972) Cutaneous polyarteritis nodosa and Crohn's disease. *Transactions of the St John's Hospital Dermatological Society*, **58**, 261.

British Journal of Dermatology (1976) **95**, 555.

Paroxysmal itching in multiple sclerosis

P.O.OSTERMAN

Department of Neurology, University Hospital, Uppsala, Sweden

Accepted for publication 29 March 1976

SUMMARY

In three women with multiple sclerosis, paroxysmal attacks of itching occurred. There were several similarities between these attacks and other types of paroxysmal phenomena previously described in multiple sclerosis. The attacks were brief, but usually lasted several minutes, they started and ended abruptly, and recurred several times a day. They were controlled effectively by carbamazepine. It is suggested that paroxysmal itching is caused by transversely spreading ephaptic activation of axons within a partially demyelinated lesion in pain-conducting fibre tracts in the central nervous system.

Itching is a rare symptom in neurological diseases. It may occur in herpes zoster and tabes dorsalis (Cairns, 1972). An itching sensation may also occasionally be observed as a sensory symptom in multiple sclerosis (MS) (McAlpine, 1972). The occurrence of paroxysmal attacks of itching in MS, however, seems to have been reported in only one patient previously (Osterman & Westerberg, 1975). This case is reviewed in the present paper (Case 2) together with two additional patients with paroxysmal itching. These three cases have several features in common.

CASE REPORTS

Case 1. A woman, born in 1922 with no relevant past history. In 1962 she had a period of numbness in both legs and right-sided optic neuritis, and in 1974 another bout of numbness in both legs. Since 1962 Lhermitte's sign (sudden, transient, electric-like shocks extending down the arms, trunk and legs on bending the head forward; due to a lesion affecting the posterior columns of the cervical cord) has been present for long periods. Neurological examination revealed nothing pathological. Agar-gel electrophoresis of the cerebrospinal fluid strengthened the diagnosis of MS.

Paroxysmal itching first occurred in 1959, over a period of 2 months, during which time she had numbness in the right thumb. The itching sensation was localized simultaneously to the right thumb, the radial part of the hand and the lateral aspect of the right upper arm. The attacks started with a creeping sensation 'beneath' the skin, followed within seconds by an intense sensation of itching. She clawed and scratched, but without relief. The attacks started and ended abruptly. They lasted 10–15 min and recurred very frequently with only short free intervals in between.

Attacks of itching recurred in 1974. The itching sensation was localized to the lateral aspects of the upper arms and the back of the neck. Most often the attacks occurred in the left arm, but sometimes both arms were affected simultaneously. When the itching occurred in the back of the neck the arms were not affected. The patient described the sensation as similar to a midge bite. The attacks started and ended abruptly. They lasted about 15 min and at first recurred about twice a week. After 6 months the frequency was one to three a day. Carbamazepine in a dosage of 200–400 mg twice daily was given in two short periods and the attacks disappeared during the treatment. In the autumn of 1975 they recurred. They now started with an itching sensation in the back of the neck, with rapid spreading to the lateral aspect of both upper arms. The itching was intense and frequently woke her up at night. An aching pain was often felt at the same time as the itching. Pinching of the itching area gave relief for about a minute. The attacks lasted up to 30 min and they recurred up to 10 times a day. Carbamazepine was given in a dosage of 200 mg three times daily and the attacks ceased completely within a day.

Case 2. A woman, born in 1940, with undoubted MS since 1962. Lhermitte's sign was present for long periods. Attacks of itching first occurred in 1962. For several months she had attacks of intense itching in the front of both thighs, starting on both sides at the same time. The itching started and ended abruptly and lasted 3–4 min. She sometimes had 15–20 attacks a day, but usually the frequency was lower.

After 1967, paroxysmal itching occurred in the hands, with periods of attacks either in the right or left hand. Within each period the attacks were always in the same hand. The itching sometimes affected the fingertips, sometimes the palm of the hand, and sometimes the whole hand. On exceptional occasions both the hand and forearm were affected. The itching sensation was described as similar to an extremely itching midge bite. The patient could not resist the urge to scratch, although this gave no relief. She scratched with hard brushes, matchboxes and knives. She put her hand in the freezer and in hot water without relief. The attacks started and ended abruptly and lasted about 3 min. Drying the hands with a towel after washing often precipitated attacks. At most she had 15 attacks a day, but usually the frequency was much lower. The patient was treated with carbamazepine from October 1971, in a dosage of 400 mg three times daily. During this treatment the attacks of itching were less frequent, shorter, and less intense. After 2 months she temporarily discontinued the treatment, and until it was resumed paroxysmal itching recurred several times a day, and was very intense. In the autumn of 1972 no attacks occurred and treatment was stopped. Subsequently there have been only sporadic attacks of slight itching.

Case 3. A woman, born in 1950, with no relevant past history. In 1972 she had right-sided optic neuritis and subsequently several bouts of numbness and weakness in the legs and arms, and a bout of vertigo. Lhermitte's sign was present for long periods. Neurological examination between the bouts revealed nothing pathological except defective colour vision of the right eye, exaggerated reflexes in the legs and abolished abdominal reflexes. Agar-gel electrophoresis of the cerebrospinal fluid strengthened the diagnosis of MS. Starting in September 1974, paroxysmal attacks of pain occurred in the ulnar part of both forearms and hands. It usually was of an aching character. The attacks lasted 2–10 min and at most they recurred 50–80 times a day. Carbamazepine 600–800 mg daily and phenytoin 300 mg daily were effective in controlling the paroxysmal pain, although sporadic attacks still occurred during this therapy. Transcutaneous electrical stimulation was tried, but had no effect. After several months the attacks became less severe and recurred less frequently.

Paroxysmal itching first occurred in November 1974. The intense itching sensation was localized to both sides of the trunk, in symmetrical areas corresponding to the thoracic spinal segments seven or eight. The attacks occurred on either side, but most often were right-sided. The itching sometimes affected the back, but was more often localized to the front of the trunk near the midline. Three days before the attacks started the patient noticed decreased sensitivity to light touch within the affected area on the right side. Within the affected areas on both sides the application of cold and pinpricking gave a hyperpathic response. The itching started abruptly but ended more gradually.

Sometimes there was a slight burning sensation at the same time. Attacks could be precipitated by light touch within the affected areas. During the attacks application of ice and firm pressure and pinching of the itching skin area gave relief for about a minute. The duration of itching was 1–10 min, and at most she had 20 attacks a day. During carbamazepine treatment (400–800 mg daily) the attacks were less frequent, shorter, and less intense. Treatment with phenytoin (serum level 52 μmol/l) seemed less effective. Transcutaneous electrical stimulation had no effect. The sensitivity in the affected areas was normalized within 6 months. Sporadic attacks still recurred, however.

In the autumn of 1975 the patient had paroxysmal itching localized to the left side of the neck. The attacks occurred during a bout of diminished sensitivity and patches of hyperaesthesia in the body below the head, weakness of the legs and urgency of micturition. Neurological examination during this bout further disclosed signs indicating disorder of the pyramidal tracts and ataxia of the right arm. The itching sensation was localized within an area, as large as the palm of a hand, in the left side of the neck. Attacks of itching could be precipitated by light touch. Firm pressure and pinching of the itching skin area mitigated the itching sensation. The itching was so intense that it disturbed the patient's sleep. The attacks lasted 15–20 min and recurred every 2–30 min. Within the same area occasional attacks of paroxysmal pain also occurred. There were also sporadic attacks of itching within small areas of the right arm. The paroxysmal itching disappeared after 1 month, concurrent with considerable improvement of the other symptoms.

DISCUSSION

The attacks of itching in these three women with multiple sclerosis have several characteristic features in common:

(1) The attacks started and ended abruptly. (2) The attacks recurred frequently, usually several times a day. (3) The duration of the attacks was usually several minutes and in no case less than 1 min. (4) In two cases the itching was localized to an area with numbness and diminished sensitivity. In these cases the distribution of the itching sensation seemed to be segmental (Case 1: cervical segment six; Case 3: thoracic segment seven or eight). (5) Two cases (2 and 3) had attacks of itching which could be precipitated by touch and two cases (1 and 3) had paroxysmal itching which was relieved by pinching of the itching skin area. (6) In all cases itching attacks occurred on both sides of the body, in symmetrical areas. (7) Carbamazepine was effective against the paroxysmal itching. (8) All three patients had itching attacks in at least two different sites during two different time periods. (9) Lhermitte's sign was present in all cases. (10) Neurological examination between the bouts revealed only minor abnormalities.

Paroxysmal symptoms are not infrequent in MS. Twenty-two of 235 patients with undoubted or suspected MS, treated at the Department of Neurology, Uppsala, during an 8-year period, had paroxysmal symptoms during the course of their disease (Osterman & Westerberg, 1975). The most common types of attacks are paroxysmal dysarthria and ataxia, and tonic seizures. Less common are paroxysmal diplopia, paroxysmal akinesia, paroxysmal numbness and paroxysmal pain. The similarities between the different kinds of paroxysmal phenomena in MS—their brevity, their stereotypical appearance in the individual patient, the frequency, the eliciting factors, the effect of phenytoin and carbamazepine—suggest that the same pathogenic mechanism is operative in the various attacks. The findings in some recent studies support the concept that paroxysmal phenomena in MS are caused by transversely spreading ephaptic activation (i.e. activation via an artificial synapse) of axons within a partially demyelinated lesion in fibre tracts somewhere in the central nervous system (Ekbom, Westerberg & Osterman, 1968; Matthews, 1975; Osterman & Westerberg, 1975).

Although there are many similarities between the various paroxysmal phenomena in MS, the duration of the different types of attacks tends to vary. Attacks of paroxysmal dysarthria and ataxia and of paroxysmal diplopia typically are brief, lasting for only a few seconds up to half a minute, and tonic seizures commonly last about half a minute. In contrast, the duration of paroxysmal pain (Espir & Millac, 1970; Matthews, 1975; present study, Case 3) and paroxysmal itching is usually several minutes. It is noteworthy that one patient (Case 3) experienced both paroxysmal pain and paroxysmal itching within the same skin area, which indicates that these two types of attacks may be related.

It is generally agreed that the peripheral and central nervous pathways subserving the sensation of itching are the same as, or closely follow, those which convey the sense of pain (Sweet, 1959; Chapman, Goodell & Wolff, 1960). The patients often compared the paroxysmal itching to an intensely itching midge bite. The itching sensation was localized 'beneath' the skin and it was sometimes associated with a burning or aching sensation. In the peripheral nerves this type of itching is considered to be mediated by unmyelinated C-fibres (Chapman *et al.*, 1960). Torebjörk (1974) recorded C-fibre activity with micro-electrodes from intact sensory fascicles in the human peroneal nerve. He found that sensations produced by cutaneous stimuli inducing intense afferent C-fibre activity were described as 'burning, or delayed pain', whereas stimuli eliciting low frequency activity were often experienced as 'itch, or burning itch'. It is therefore possible that irritative lesions in pain-conducting fibre pathways may cause both paroxysmal pain and paroxysmal itching—differences in impulse patterns determining whether the sensation will be one of itch or of pain. The frequent occurrence of itching attacks in symmetrical areas on both sides of the body suggests that the irritative lesion in these cases may be located in the spinal cord, affecting the decussating spinothalamic nerve fibres. The occurrence of itching with a segmental distribution in two cases also indicates that paroxysmal itching may have a spinal origin.

Carbamazepine is generally considered to be the most valuable drug for treatment of paroxysmal phenomena in MS and it was effective in controlling the paroxysmal itching in all three cases. In itch due to various disorders, transcutaneous electrical nerve stimulation has been reported to be effective in many cases (Carlsson *et al.*, 1975). This was only tried in one of the three present cases and had no effect on the paroxysmal itching.

Finally, it is worthy of mention that none of the patients thought of a connexion between the paroxysmal itching and their neurological disease. Two of them had consulted physicians and general practitioners about the attacks. Furthermore, paroxysmal itching may be one of the earliest symptoms of MS. It is therefore conceivable that other patients with paroxysmal itching may consult physicians other than neurologists for their attacks of itching.

REFERENCES

CAIRNS, R.J. (1972) The skin and the nervous system. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S. Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1791. Blackwell Scientific Publications, Oxford.

CARLSSON, C.-A., AUGUSTINSSON, L.-E., LUND, S. & ROUPE, G. (1975) Electrical transcutaneous nerve stimulation for relief of itch. *Experientia*, **31**, 191.

CHAPMAN, L.F., GOODELL, H. & WOLFF, H.G. (1960) Structures and processes involved in the sensation of itch. In: *Advances in Biology of Skin. Cutaneous Innervation* (Ed. by W.Montagna), Vol. 1, p. 161. Pergamon Press, Oxford.

EKBOM, K.A., WESTERBERG, C.-E. & OSTERMAN, P.O. (1968) Focal sensory-motor seizures of spinal origin. *Lancet*, **i**, 67.

ESPIR, M.L.E. & MILLAC, P. (1970) Treatment of paroxysmal disorders in multiple sclerosis with carbamazepine (Tegretol). *Journal of Neurology, Neurosurgery, and Psychiatry*, **33**, 528.

MCALPINE, D. (1972) Symptoms and signs. In: *Multiple Sclerosis. A Reappraisal* (Ed. by D.McAlpine, C.E. Lumsden and E.D.Acheson), 2nd edn, p. 132. Williams and Wilkins, Baltimore.

MATTHEWS, W.B. (1975) Paroxysmal symptoms in multiple sclerosis. *Journal of Neurology, Neurosurgery, and Psychiatry*, **38**, 617.

OSTERMAN, P.O. & WESTERBERG, C.-E. (1975) Paroxysmal attacks in multiple sclerosis. *Brain*, **98**, 189.

SWEET, W.H. (1959) Pain. In: *Handbook of Physiology. Sect. 1. Neurophysiology* (Ed. by J.Field, H.W.Magoun and V.E.Hall), Vol. 1, p. 459. American Physiological Society, Washington D.C.

TOREBJÖRK, H.E. (1974) Afferent C units responding to mechanical, thermal and chemical stimuli in human non-glabrous skin. *Acta Physiologica Scandinavica*, **92**, 374.

British Journal of Dermatology (1976) **95**, 559.

Generalized warts and immune deficiency

T.M.S.REID, N.G.FRASER* AND I.R.KERNOHAN†

Department of Bacteriology, University of Aberdeen
and *Departments of Dermatology and †Surgery, Aberdeen Royal Infirmary

Accepted for publication 5 April 1976

SUMMARY

A case of common variable hypogammaglobulinaemia with associated impairment of cell mediated immunity and severe wart virus infection is described. The defect of cell mediated immunity is thought to have predisposed this patient to the development of persistent wart infection which in turn grossly depressed the body's cellular immunity and thus allowed widespread dissemination of the warts. The rapid restoration of cell mediated immunity which followed the reduction in the antigenic load of wart virus by diathermy treatment was followed by the spontaneous regression of all the patient's warts. This unusual case may provide some insight into the complex relationship between wart virus infection and the immune system of the host.

There is much debate about the relative importance of antibodies and cell mediated immunity in the spontaneous regression of human warts. The occurrence of humoral antibody to human wart virus is well documented (Almeida & Goffe, 1965; Ogilvie, 1970; Genner, 1971; Bunney *et al.*, 1971; Pyrhonen & Penttinen, 1972; Pass & Maizel, 1973; Matthews & Shirodaria, 1973) but as yet no significant correlation between antibody levels and wart regression has been demonstrated.

Evidence for the involvement of cell mediated immunity in wart regression has come from observations that patients with immunodeficiencies have an increased incidence of warts (Spencer & Andersen, 1970; Koranda *et al.*, 1974; Perry & Harman, 1974) and Morison (1975a) noted that those patients with a predominantly cell mediated or a mixed antibody and cell mediated defect were susceptible in contrast to those with pure antibody defects. A recent study of the immunocompetence of wart patients revealed a relative deficiency of cell mediated immunity which appeared to be related to the duration of infection (Morison, 1975b) thus confirming the observations of Brodersen, Genner & Brodthagen (1974) that children with warts showed reduced tuberculin reactivity when compared to non-affected controls. In addition the observation that a marked dermal infiltrate of mononuclear cells is present around spontaneously involuting warts (Biberstein, 1944; Tagami *et al.*, 1974) suggests a role for cell mediated immunity in wart regression.

CASE REPORT

A.S., a 19-year-old male, first attended the Aberdeen Skin Clinic in August 1973 with a 13-year history of warts. The warts had originally appeared on the hands and knees, but over the previous 6 years the soles of the feet had been involved and for the 2 years prior to being seen the warts had increased in size and number and had spread to the face and upper chest. The patient latterly had frequently to discontinue his work as an apprentice plumber because of bleeding and pain in the warts on his hands. Local treatment of the warts, supervised in another part of the country, had not produced any benefit. Otherwise, the patient was well and in particular had not noted any increased susceptibility to infection. One of the patient's five brothers had had warts in childhood but these had cleared spontaneously.

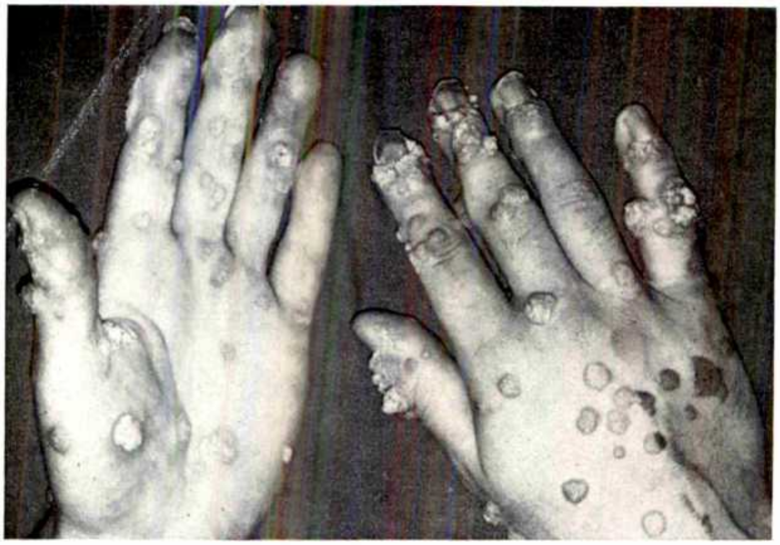


FIGURE 1.

Examination revealed extensive common warts on the hands (Fig. 1) and knees. Plantar warts were also present and these, as well as the hand lesions, were markedly hypertrophic. Filiform warts were noted on the face, neck and upper chest. No other dermatological abnormalities were observed, and general examination was negative.

The hand warts were treated by diathermy under general anaesthesia on four separate occasions over a period of approximately 2 months and when the patient was seen for review 1 month later the remaining warts in all areas were regressing spontaneously. During the first course of treatment a lymph node was biopsied and a specimen of wart was removed for electron microscopic studies.

On follow-up the patient remained clear for 2 years before developing further warts on the penis and in the perianal area. These were also treated by diathermy under general anaesthesia.

Routine investigations. Hb 16·3 g/100 ml; W.B.C. 5600/mm^3 with 14% lymphocytes; E.S.R. 2 mm in 1 h; Liver function tests normal. Total serum proteins 4·8 g/100 ml, albumin 2·8 g/100 ml, globulin 2·0 g/100 ml. Urinalysis normal. Blood urea and electrolytes normal. Chest X-ray normal.

Immunological investigations. The full range of skin tests and lymphocyte transformation studies were performed prior to the start of treatment. Two years after the completion of treatment only the PPD (1 I.U.) skin test and the PHA lymphocyte stimulation were repeated. The assessment of levels of serum immunoglobulins, wart specific antibody and circulating T and B lymphocytes were performed on five occasions as detailed in Tables 1 and 2.

MATERIALS AND METHODS

Electron microscopy. Specimens of the excised warts were prepared and examined in the electron microscope after negative staining (Almeida, Cinader & Howatson, 1963) for the presence of wart virus particles.

TABLE 1. Serum immunoglobulin levels before, during and after diathermy treatment

	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	IgE (U/ml)
Pre-treatment	350	40	42	130
2 weeks after start of treatment	590	65	35	124
6 weeks after start of treatment	350	65	35	190
4 weeks after completion of treatment	420	80	40	130
2 years after treatment	405	54	40	155
Normal range	600–1800	115–430	50–190	30–700

TABLE 2. Numbers of T and B lymphocytes before, during and after treatment

	T cells (%)	B cells (%)
Pre-treatment	10	30
2 weeks after start of treatment	34	28
6 weeks after start of treatment	45	30
4 weeks after completion of treatment	57	35
2 years after treatment	42	41
Normal range	40–60	25–35

Serum immunoglobulins G, A and M were measured using 'Hyland' immunodiffusion plates. IgE was estimated by radio-immunoassay (Phadebas).

Detection of specific antibody to wart antigen. Immunodiffusion tests were performed according to the method of Almeida & Goffe (1965) using the serum from a rabbit immunized against wart virus as a positive control.

Skin testing. Delayed hypersensitivity skin tests were performed using PPD 1, 10 and 100 i.u. and 0·1 ml *Candida albicans* (100 μg/ml) and mumps antigen (Eli Lilly). Contact sensitivity to dinitrochlorobenzene (DNCB) was assessed by sensitizing the skin with 0·1 ml of 5% solution in acetone followed by a challenge 1 week later with 0·1 ml of 0·1% DNCB in acetone. The reaction was observed over 72 h.

T and B cells. T-lymphocytes were identified by their ability to form spontaneous rosettes with sheep red blood cells (Jondal, Holm & Wigzell, 1972) and B lymphocytes by their ability to form rosettes with C3 coated red cells (Bianco, Patrick & Nussenzweig, 1970).

Lymphocyte stimulation. Peripheral blood lymphocytes isolated from fresh defibrinated blood by the Ficoll–Triosil method were suspended at a concentration of 10^6 cells/ml in membrane filtered hepes-buffered RPMI 1640 medium (Flow) pH 7·4 supplemented with glutamine (2mM), penicillin (100 units/ml), streptomycin 0·1 mg/ml and fetal calf serum (10%). Duplicate cultures (0·2 ml) were incubated in flat bottomed tissue culture plates (Falcon) at 37°C in a humidified atmosphere (5% CO_2 in air). The following mitogens were used, control cultures receiving medium only; phytohaemagglutinin (PHA, Wellcome) 1 μg/ml, concanavalin A (CON A, Sigma) 10 μg/ml, pokeweed mitogen (PWM, G.I.B.C.O.) 2 μg/ml and PPD (Ministry of Agriculture, Weybridge) 5 μg/ml. After 46 h incubation or 5 days in the case of PWM DNA synthesis was assessed by adding 1 μCi tritiated thymidine (Radiochemical Centre, Amersham) to each well. Two hours later the cells were harvested with the multiple cell harvester (Flow Laboratories) and the radioactivity incorporated measured in a liquid scintillation counter, results being expressed as counts per minute.

RESULTS

Throughout the period of study the levels of IgG, A and M were below the normal range (Table 1) while the IgE levels were normal. Antibodies specific for human wart virus antigen were not detected in any of the serum samples.

Rosetting studies (Table 2) revealed a much decreased percentage of circulating T lymphocytes at the outset. There was a steady recovery in the percentage of T cells following treatment and throughout the period of spontaneous regression until essentially normal values were obtained. Levels of circulating B cells were normal.

The results of lymphocyte transformation studies are shown in Table 3. The patient responded poorly to PHA, CON A and PWM but showed normal reactivity to PPD. Data from a 'normal' age and sex matched control whose cells were cultured at the same time are included for comparison. Testing performed 2 years after regression of warts showed a normal response to PHA.

The patient demonstrated total intradermal anergy by failing to react to any of the test antigens and in addition he could not be sensitized to DNCB.

Biopsy of a lymph node draining an area of wart involvement revealed the following abnormalities. (a) Marked depletion of lymphocytes in the paracortex with prominent reticulum cells. (b) poor development of germinal centres.

TABLE 3. Tritiated thymidine incorporation into stimulated lymphocytes (counts/min)

	Patient		Control
	Pre-treatment	2 years post-treatment	
Control	733	869	620
PHA	9,611	37,524	47,434
CON A	3,051	—	30,784
PWM	8,311	—	20,938
PPD	65,028	—	52,311

Specimens of the warts examined in the electron microscope were found to contain many virus particles.

DISCUSSION

It is clear that this patient suffered from a form of common variable hypogammaglobulinaemia (Cooper *et al.*, 1974). About a third of such cases have demonstrable evidence of some depression in cell mediated immunity which although not usually associated with clinical problems is likely to have predisposed this patient to develop severe intractable wart virus infection (Spencer & Andersen, 1970, Koranda *et al.*, 1974; Morison, 1975a). Matthews & Shirodaria (1973) noted that long standing non-regressing warts of this type often lack demonstrable wart virus and are often found in patients with low or absent levels of anti-wart virus specific antibody. Paradoxically, however, multiple virus particles were easily visualized in this case but wart virus specific antibodies were not detectable at any time during the infection and in particular during the phase of spontaneous regression. This is an important finding as it may imply a major role for cell mediated immunity in regression of this patient's warts.

The lymphocyte transformation studies showed a depression of responsiveness particularly to the pure T cell mitogens PHA and CON A (Table 3). Addition of human wart virus to lymphocyte cultures has been shown to depress lymphocyte transformation *in vitro* (Willems, Melnick & Rawls, 1969) but direct evidence of *in vivo* depression of cell mediated immunity has been lacking. A possible explanation for the depressed lymphocyte transformation is to be found in the low level of circulating T rosettes present prior to commencing treatment. This finding may be directly related to the common variable hypogammaglobulinaemia or more likely, as Wybran & Fudenberg (1973) demonstrated, it may represent a depression induced by the viral infection itself which resolved on recovery from the infection (Table 2).

There are many obvious parallels between the present case and the immunological spectrum found in leprosy. Thus the findings in the present case of impaired cell mediated immunity evidenced by reduced circulating T cells, depressed lymphocyte transformation and complete skin test anergy, and the changes in lymphoid tissue seen in the distortion and lymphocyte depletion of the lymph node paracortex are also well described in lepromatous leprosy (Turk & Waters, 1971). In both conditions a paradox exists between the apparently non-specific depression in cell mediated immunity and the observed competence of the host in resisting other infections (Turk & Waters, 1968). Also studies by Nath *et al.* (1974) in lepromatous leprosy have shown that the depression in T rosettes correlates well with the mycobacterial load in these patients and that after treatment of the infection levels of T rosettes return to normal. Similarly the recovery of T rosettes in this patient followed the reduction of the wart virus antigen load by diathermy treatment.

Finally it can be postulated that the restoration of cell mediated immunity which occurred in this case after treatment resulted in the rapid elimination of the residual wart virus infection.

REFERENCES

ALMEIDA, J., CINADER, B. & HOWATSON, A. (1963) The structure of antigen–antibody complexes. A study by electron microscopy. *Journal of Experimental Medicine*, **118**, 327.

ALMEIDA, J.D. & GOFFE, A.P. (1965) Antibody to wart virus in human sera demonstrated by electron microscopy and precipitin tests. *Lancet*, **ii**, 1205.

BIANCO, C., PATRICK, R. & NUSSENZWEIG, V. (1970) Population of lymphocytes bearing a membrane receptor for antigen–antibody–complement complexes. *Journal of Experimental Medicine*, **132**, 702.

BIBERSTEIN, H. (1944) Immunisation therapy of warts. *Archives of Dermatology and Syphilology*, **50**, 12.

BRODERSEN, I., GENNER, J. & BRODTHAGEN, H. (1974) Tuberculin sensitivity in BCG-vaccinated children with common warts. *Acta dermato-venereologica*, **54**, 291.

BUNNEY, M.H., HUNTER, J.A.A., OGILVIE, M.M. & WILLIAMS, D.A. (1971) The treatment of plantar warts in the home. A critical appraisal of a new preparation. *Practitioner*, **207**, 197.

COOPER, M.D., FAULK, W.P., FUDENBERG, H.H., GOOD, R.A., HITZIG, W., KUNKEL, H.G., ROITT, I.M, ROSEN, F.S., SELIGMANN, M. & SOOTHILL, J.F. (1974) Meeting report of the second international workshop on primary immunodeficiency diseases in man. *Clinical Immunology and Immunopathology*, **2**, 416.

GENNER, J. (1971) Verrucae vulgares. II. Demonstration of a complement fixation reaction. *Acta dermato-venereologica*, **51**, 365.

JONDAL, M., HOLM, G. & WIGZELL, H. (1972) Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *Journal of Experimental Medicine*, **136**, 207.

KORANDA, F.C., DEHMEL, E.M., KAHN, G. & PENN, I. (1974) Cutaneous complications in immunosuppressed renal homograft recipients. *Journal of the American Medical Association*, **229**, 419.

MATTHEWS, R.S. & SHIRODARIA, P.V. (1973) Study of regressing warts by immunofluorescence. *Lancet*, **i**, 689.

MORISON, W.L. (1975a) Viral warts, herpes simplex and herpes zoster in patients with secondary immune deficiencies and neoplasms. *British Journal of Dermatology*, **92**, 625.

MORISON, W.L. (1975b) Cell-mediated immune responses in patients with warts. *British Journal of Dermatology*, **93**, 553.

NATH, I., CURTIS, J., BHUTANI, L.K. & TALWAR, G.P. (1974) Reduction of a subpopulation of T lymphocytes in lepromatous leprosy. *Clinical and Experimental Immunology*, **18**, 81.

OGILVIE, M.M. (1970) Serological studies with human papova (wart) virus. *Journal of Hygiene*, **68**, 479.

PASS, F. & MAIZEL, J.V. (1973) Wart-associated antigens. II. Human immunity to viral structural proteins. *Journal of Investigative Dermatology*, **60**, 307.

PERRY, T.L. & HARMAN, L. (1974) Warts in diseases with immune defects. *Cutis*, **13**, 359.

PYRHONEN, S. & PENTTINEN, K. (1972) Wart-virus antibodies and the prognosis of wart disease. *Lancet*, **ii**, 1330.

SPENCER, E.S. & ANDERSEN, H.K. (1970) Clinically evident, non terminal infections with herpes viruses and the wart virus in immunosuppressed renal allograft recipients. *British Medical Journal*, **iii**, 251.

TAGAMI, H., OGINO, A., TAKIGAWA, M., IMAMURA, S. & OFUJI, S. (1974) Regression of plane warts following spontaneous inflammation. An histopathological study. *British Journal of Dermatology*, **90**, 147.

TURK, J.L. & WATERS, M.F.R. (1968) Immunological basis for depression of cell mediated immunity and the delayed allergic response in patients with lepromatous leprosy. *Lancet*, **ii**, 436.

TURK, J.L. & WATERS, M.F.C. (1971) Immunological significance of changes in lymph nodes across the leprosy spectrum. *Clinical and Experimental Immunology*, **8**, 363.

WILLEMS, F.Th.C., MELNICK, J.L. & RAWLS, W.E. (1969) Viral inhibition of the phytohaemagglutinin response of human lymphocytes and application to viral hepatitis. *Proceedings of the Society for Experimental Biology and Medicine*, **130**, 652.

WYBRAN, J. & FUDENBERG, H.H. (1973) Thymus-derived rosette-forming cells in various human disease states: cancer, lymphoma, bacterial and viral infections and other diseases. *Journal of Clinical Investigation*, **52**, 1026.

British Journal of Dermatology (1976) **95**, 565.

Brief Communications

Improved technique for epidermal cell cycle analysis

F.W.Bauer *and* R.M.de Grood *Department of Dermatology, University of Nijmegen, Nijmegen, The Netherlands*

We have recently described the application of pulse cytophotometry to epidermis (Bauer & de Grood, 1975). Further experience with this technique has led to certain improvements in the method of sample preparation and staining, which we report here.

Biopsies (average 2–3 mm diameter, 0·3 mm depth) are derived by pressing a thin strip of metal on the skin. The skin bubbles up through a 5 mm diameter hole in the metal. The projecting skin is removed with a razor blade (Fig. 1) and incubated for 15 min at 37°C in a balanced salt solution containing 1% trypsin and 0·3% dithioerythrol (Hentzer & Kobayasi, 1976). The intact specimens are transferred to a centrifuge tube containing 5 ml of propidium iodide (1 mg/100 ml) in 0·3 % sodium citrate. One minute of vigourous stirring on a 'Vortex' mixer is now sufficient to detach the epidermis from the dermis and to dissociate the epidermal cells which are simultaneously stained, since the cell

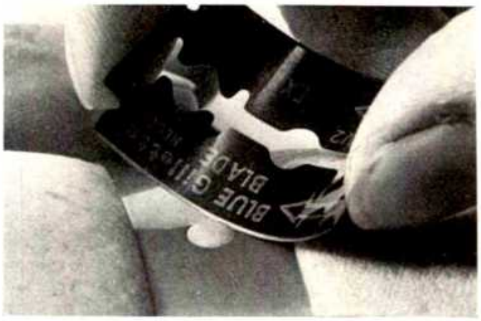


FIGURE 1.

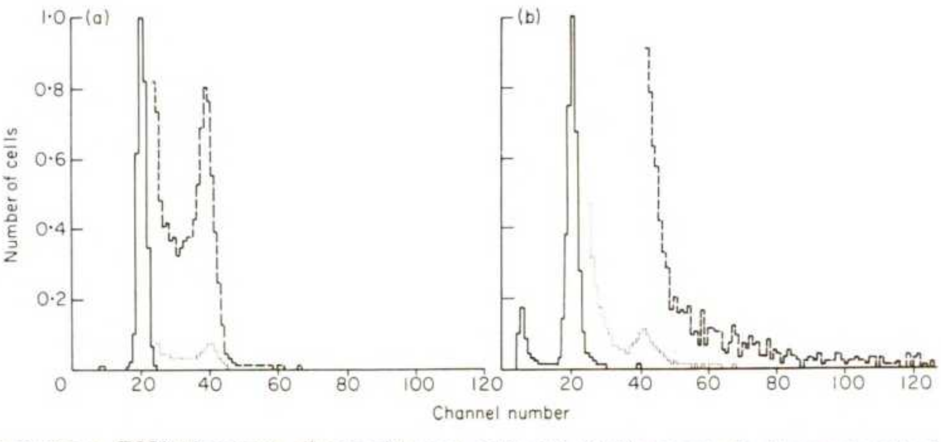


FIGURE 2. DNA histograms of normal human epidermis. (a) Represents the improved method, (b) the old method.

...... 10 fold amplification of the solid line; - - - - - 100 fold amplification of the solid line.

membranes have been rendered permeable for the fluorochrome by the hypotonic citrate solution (Krishnan, 1975). After filtration through nylon gauze a few drops of RNase (10 mg/ml) are added to remove extra-nuclear staining, and the cell suspension is ready for measurement.

Fig. 2 shows a typical histogram obtained with this modified technique in comparison to that obtained using the method described previously. Advantages include the following:

(1) The total time required for sample preparations is reduced from 3 h to 30 min.

(2) Cell loss is minimal since no centrifugation steps are required.

(3) Agglutination is minimal; even after × 100 amplification of the original signal, clumps of three cells can hardly be detected.

(4) The variation coefficient (that is the width of the histogram) is reduced.

REFERENCES

BAUER, F.W. & DE GROOD, R.M. (1975) Impulse cytophotometry in psoriasis. *British Journal of Dermatology*, **93**, 225.

HENTZER, B. & KOBAYASI, T. (1976) Dissociation of human adult epidermal cells by disulfide-reducing agents and subsequent trypsinization. *Acta dermato-venereologica*, **56**, 19.

KRISHAN, A. (1975) Rapid flow cytofluorometric analysis of mammalian cell cycle by propidium iodide staining. *The Journal of Cell Biology*, **66**, 188.

Effect of hydrocortisone butyrate on collagen synthesis

HEIKKI SAARNI *and* VÄINÖ K. HOPSU-HAVU *Departments of Medical Chemistry and Dermatology, University of Turku*, 20520 *Turku* 52, *Finland*

The more potent the anti-inflammatory steroid used in local therapy the greater is the likelihood of undesirable side effects such as thinning of the skin, striae, telangiectases and aggravation of rosacea and perioral dermatitis. Topical hydrocortisone is considered very safe but relatively ineffective. The fluorinated corticosteroids are very effective but are known to have a greater incidence of side effects. In an effort to combine the safety of hydrocortisone and the efficacy of the fluorinated steroids, hydrocortisone butyrate and other hydrocortisone derivatives have been synthesized. This new steroid has been shown to be effective in several clinical trials (Polano *et al.*, 1970, 1973; Ashurst, 1972; Alexander, 1973) and is said to be devoid of the clinical side effects of fluorinated steroids. Because hydrocortisone butyrate did not prevent recovery from atrophy of the face induced by treatment with fluorinated steroids, Sneddon (1973) concluded that its use in conditions such as psoriasis and eczema would not cause dermal atrophy.

This paper reports our findings on the effects of hydrocortisone butyrate on collagen synthesis measured as incorporation of proline into hydroxyproline by chick embryo fibroblasts compared with the effects of hydrocortisone succinate, prednisolone and betamethasone valerate.

Tendon cells from 17-day-old chicken embryos were isolated by the method of Dehm & Prockop as slightly modified by Rönnemaa *et al.* (1975). The cells were suspended in modified Krebs-Ringer medium (141 mM NaCl, 5·6 mM KCl, 3·0 mM $CaCl_2$, 1·4 mM KH_2PO_4, 1·4 mM $MgSO_4$, 22 mM glucose) buffered with 20 mM N-2-hydroxyethylpiperazine-N^1-2-ethansulphonic acid (HEPES, Sigma), pH 7·4, containing 0·015% bovine serum albumin (A 4503, Sigma), 0·1 mM ascorbic acid and 14·5 uM L-proline.

The incubations were performed in 25 ml polypropylene tubes containing between 2×10^6 and 3×10^6 cells as determined with an automatic cell-counter using 3 ml of medium. Incubations were carried out in the absence and in the presence of the following steroids: hydrocortisone butyrate (Gist-Brocades, Delft, Holland), hydrocortisone-Na-succinate (Orion, Helsinki, Finland), prednisolone (Merck, Darmstadt, Germany) and betamethasone-17-valerate (Glaxo, Greenford, England).

After a preliminary incubation for 15 minutes at 37°C, 5μCi of L(G-^{3}H)-proline (TRA 82, Batch No. 12, Radiochemical Centre, Amersham, England) was added to the medium. The incubation time was 120 min with air as the gas phase. The incubations were stopped by cooling the tubes to 0°C in an ice bath and by adding 0·27 mg of cyclohexidine (Sigma) and 0·4 mg of alfa-alfa-dipyridyl (Merck) in 0·2 ml of medium. The viability of the cells was confirmed by the trypan blue test.

After incubation the cells were separated by centrifuging at 350 *g* for 12 min. The cell pellet was washed, suspended in distilled water and sonicated. The homogenates and media were dialyzed against tap water overnight and the amount of labelled hydroxyproline was measured by the method of Juva & Prockop (1966).

The results are shown in Fig. 1. At a concentration of 10^{-5} M a slight stimulation of collagen synthesis was observed with hydrocortisone succinate. Hydrocortisone butyrate and prednisolone caused a slight but significant inhibition. Betamethasone-17-valerate caused greater inhibition. At a concentration 10^{-4} M hydrocortisone succinate had no clear effect while hydrocortisone butyrate and prednisolone caused an inhibition of about 20–30%. Betamethasone-17-valerate caused an inhibition of proline incorporation into collagen of approximately 90%.

The tendon cells used in this study provide a suitable system for the study of collagen synthesis because about 80% of the total protein synthesis is collagen and 70–80% of the radioactive proline incorporated is found in collagen (Dehm & Prockop, 1971). The results clearly show that hydrocortisone succinate has relatively little effect on collagen synthesis, even at a concentration of 10^{-4} M. A similar finding was reported by Uitto & Mustakallio (1971). Hydrocortisone butyrate and prednisolone at a concentration of 10^{-4} M cause an approximately equal inhibition of collagen synthesis. Betamethasone-17-valerate was by far the most potent of the steroids tested, producing approximately 3 × greater inhibition than prednisolone and hydrocortisone butyrate.

On the basis of these *in vitro* findings one would expect that the harmful effects of hydrocortisone butyrate on skin collagen synthesis in local use would be of the same order as those of prednisolone, i.e. closer to the effects of hydrocortisone succinate than to those of betamethasone-17-valerate.

REFERENCES

Alexander, S. & Lyne, C. (1973) A preliminary clinical trial of hydrocortisone-17-butyrate. *British Journal of Clinical Practice*, **27**, 177.

Ashurst, P.J. (1972) Hydrocortisone-17-butyrate, a new synthetic topical corticosteroid. *British Journal of Clinical Practice*, **26**, 263.

Dehm, P. & Prockop, D.J. (1971) Synthesis and extrusion of collagen by freshly isolated cells from embryo tendon. *Biochimica et Biophysica Acta*, **240**, 358.

Juva, K. & Prockop, D.J. (1966) Modified procedure for the assay of ^{3}H or ^{14}C-labelled hydroxyproline. *Annals of Biochemistry and Experimental Medicine*, **15**, 77.

Polano, M.K. & Kanaar, P. (1973) A clinical trial with hydrocortisone butyrate cream in eczema. *British Journal of Dermatology*, **88**, 83.

Polano, M.K., Suurmond, D., v.d. Lely, M.A. & Warnaar, P. (1970) A clinical trial with hydrocortisone butyrate cream in psoriasis. *British Journal of Dermatology*, **83**, Jubilee issue, 93.

Rönnemaa, T., Juva, K. & Kulonen, E. (1975) Effect of hyperlipidemic rat serum on the synthesis of collagen by chick embryo fibroblasts. *Arteriosclerosis*, **21**, 315.

Sneddon, I. (1973) A trial of hydrocortisone butyrate in the treatment of rosacea and perioral dermatitis. *British Journal of Dermatology*, **89**, 505.

Uitto, J. & Mustakallio, K. (1971) Effect of hydrocortisone acetate, fluocinolone acetonide, betamethasone-17-valerate and fluprednyliden-21-acetate on collagen biosynthesis. *Biochemical Pharmacology*, **20**, 2495.

PUVA-induced bullous pemphigoid

KRISTIAN THOMSEN *and* HENNING SCHMIDT *Departments of Dermatology, Finsen Institute, Copenhagen, and Odense Hospital, Odense, Denmark*

The distribution of lesions in pemphigus erythematosus is ascribed to the adverse effect of sunlight on this type of pemphigus. It has been shown that a positive Nikolsky-sign could be provoked by exposure to an erythema-producing dose of UV-light (Cram & Winkelmann, 1965). UV-light of wave lengths shorter than 320 nm applied to the normal skin of six patients, four with pemphigus vulgaris and two with bullous pemphigoid, produced fresh histological and immunohistochemical changes in the irradiated sites (Cram & Fukuyama, 1972).

This report concerns the provocation of bullous pemphigoid in two psoriatics during oral treatment with 8-methoxypsoralen (8-MOP) and UVA (PUVA therapy).

Case 1

A 57-year-old man had had psoriasis from the age of 28 and bullous pemphigoid clinically diagnosed at the age of 54. The diagnoses were confirmed histologically and by immunofluorescence which showed a band-like distribution of anti-IgG and anti-C_3 at the basal membrane. Azathioprine controlled the bullous disease and for 6 months before the present episode the patient was without treatment. However, following 8-MOP 50 mg orally and UVA given every other day small blisters developed on the upper arms after three treatments. Histologically a subepidermal bulla was found and by immunofluorescence anti-IgG and anti-C_3 were seen as a homogeneous band at the basal membrane of the involved skin. Reducing the intensity of photochemotherapy succeeded in clearing his psoriasis despite the continuous appearance of smaller and larger bullae. Blisters first appeared on the shoulders and upper arms, then on the buttocks and thighs, and finally on the legs, with a tendency to disappear in the same order. Finally, PUVA was discontinued, but even then large bullae continued to develop during the next 3 weeks, to involve most of the skin surface, leaving large infected erosions. Treatment with prednisone 100 mg and azathioprine 200 mg daily was re-instituted and controlled the bullous pemphigoid after another week.

Case 2

A 68-year-old man had had psoriasis from the age of 17, previously treated with tar preparations, topical corticosteroids and, up to 1 year before the present admission, with methotrexate in weekly doses of 15 mg to a total dose of 405 mg. For the last 4 weeks before the start of PUVA-treatment he received sulphamethoxazole-trimethoprim (Bactrim), two tablets twice a day for an upper respiratory tract infection.

PUVA-treatment with 30 mg 8-MOP 4 times a week was started. Following the fourth treatment burning, itching and redness were observed and the treatment was discontinued. However, 5 days later marked redness and infiltration of the skin were still present and small tense blisters had developed. Fresh blisters on the extremities, all arising on normal skin, continued to appear for a further week but without therapy the eruption then stopped.

By immunofluorescence anti-IgG and anti-C_3 were found as a homogeneous band at the basal membrane in the involved skin. Histologically a subepidermal bulla with some eosinophils was seen and the blood eosinophil count was 19%. No further provocation with PUVA or the sulphonamide was performed, but it should be stressed that Bactrim had been given during an extremely sunny period with no side effects at all.

COMMENT

Both patients developed a bullous disease diagnosed as bullous pemphigoid after a few exposures to PUVA, utilizing the standard dosage for psoriasis. Case 1 had a previous diagnosis of bullous pemphigoid, albeit quiescent at the time of PUVA-treatment, and it is quite evident that PUVA was the cause of the relapse. Case 2 is less clear. The patient had received sulphamethoxazole-trimethoprim immediately before treatment with PUVA was instituted and therefore it cannot be completely ruled out that the bullous pemphigoid was caused by the sulphonamide and not the PUVA; however, previous intense sun exposure had done him no harm.

Bullous pemphigoid has been described as developing following therapy with salicylazosulphapyridine (Bean, Good & Windhorst, 1970) but that patient was treated also with diphenyloxylate and atropine. Recently frusemide in standard dosage has also been reported to be able to provoke bullous pemphigoid, confirmed by immunofluorescence tests (Fellner & Katz, 1976). However, this report points to an additional side effect of the PUVA-treatment. The near future may reveal the frequency of this side effect and perhaps tell us if the association of psoriasis and bullous pemphigoid is a mere coincidence.

REFERENCES

Bean, S.F., Good, R.A. & Windhorst, D.B. (1970) Bullous pemphigoid in an 11-year-old boy. *Archives of Dermatology*, **102**, 205.

Cram, D.L. & Winkelmann, R.K. (1965) Ultraviolet-induced acantholysis in pemphigus. *Archives of Dermatology*, **92**, 7.

Cram, D.L. & Fukuyama, K. (1972) Immunohistochemistry of ultraviolet-induced pemphigus and pemphigoid lesions. *Archives of Dermatology*, **106**, 819.

Fellner, M.J. & Katz, J.M. (1976) Occurrence of bullous pemphigoid after furosemide therapy. *Archives of Dermatology*, **112**, 75.

British Journal of Dermatology (1976) **95**, 571.

Theses

In vitro studies of antagonism among human skin bacteria. P. D. MARSH (1975) Ph.D. Thesis, University of London.

Strains of coagulase negative *Staphylococcus epidermidis* isolated from human skin have been identified as producing an 'antibiotic' which has been partially characterized as a low molecular weight peptide substance having the power to prevent or restrict the growth of other skin organisms including strains of *Staphylococcus*.

The ability of this strain to grow with, and antagonize, other bacteria has been tested on solid growth media under laboratory conditions. The producer strain, designated S6 +, was active against all Gram positive species tested but had a limited activity against Gram negative species. Under some conditions the producer strain was itself antagonized by streptococcus species. Chemostat cultures showed that S6 + was usually able to inhibit other competing strains added either before or after S6 +, i.e. the producer strain was able to compete and establish itself rapidly even in the presence of a dense pre-existing culture in the chemostat. The ecological significance of this 'antibiotic' production is discussed.

W.C.NOBLE

Studies on interactions of human skin micro-organisms on lid surfaces. RAJAA M. M. MILYANI (1976) Ph.D. Thesis, University of London.

Quantitative studies of growth kinetics of skin micro-organisms were carried out using solid growth media. Amongst the strains investigated was the antibiotic-producing strain of *Staphylococcus epidermidis* 'S6 +' used by Marsh. On solid media the inhibitory action of this strain was more marked than in fluid media perhaps because of the dilution factor inherent in chemostat studies. Media designed more closely to resemble the chemical composition of the epidermis gave similar results in general but one strain of *Micrococcus sp* M7, formerly very sensitive to the antibiotic produced by S6 +, proved refractory on this medium and indeed was able to inhibit the growth of S6 + itself. This must have implications for any direct attempt to infer ecological behaviour from laboratory findings.

The media representing epidermal constituents had one other noteworthy effect on members of the cutaneous flora, for they were capable of greatly modifying the morphology of the diphtheroids. When the concentration of amino acids was 480 mg/l or less the diphtheroids were virtually coccoid whilst at concentrations above 740 mg/l morphology was the 'normal' pleomorphic rod shape. (However on the skin surface amino acids concentration in 'sweat' may reach 1400 mg/l and diphtheroids appear filamentous under the scanning electron microscope).

Milyani has progressed some way to producing an apparatus which will permit continuous observation of growth on solid surfaces; this may prove invaluable when fully developed.

W.C.NOBLE

Acne vulgaris: a laboratory and clinical study with special reference to microbial factors in pathogenesis. S. G. TAN (1976) M.D. Thesis, University of Leeds.

Cornybacterium acnes is regarded as the micro-organism most likely to be involved in the pathogenesis of acne. *C. acnes* resides in the pilosebaceous duct and is known to produce various exoenzymes; the most extensively studied of these enzymes is the lipase which releases irritant free fatty acids from sebum triglycerides. *C. acnes* lipase has been assayed using a modification of the autoburette titration using Doles extraction procedure. Optimal conditions for lipase activity on triolein *in vitro* are pH 7·0, temperature 37°C and $10^{-3}Ca^{++}$ or Mg^{++}. *C. acnes* hyaluronidase was also studied using Reissig's method for determination of n-acetylglucosamine; optimal conditions were pH 6·1 and temperature 38°C.

Antilipase and antihyaluronidase activity was investigated. Protease measured on azocasein was not wholly satisfactory owing to the small quantities of enzyme present.

Unfractionated supernatant from *C. acnes* cultured *in vitro* at pH 5·6 contained lipase, hyaluronidase and other, uncharacterized, enzymes. When applied to the skin of patients with moderate to severe acne an inflammatory reaction resulted.

These studies reinforce the view that the micro-environment within the pilosebaceous duct must be investigated in detail before a rationale for acne can be deduced.

W.C.Noble

Book Reviews

Allergic Drug Reactions. H.E. Amos (1976) London: Edward Arnold. Pp. 136, black/white illustrations.

The standard reference work on adverse actions of drugs is Meyler's 'Side Effects of Drugs', and it is disconcerting to see the way in which each edition has steadily increased in size until now with the eighth edition—'A Survey of Unwanted Effects of Drugs Reported 1972–1975'—we are faced with a compilation extending over more than 1100 pages. Equally disconcerting is our ignorance of the pathogenesis of drug reactions in many instances. Meyler and several similar tomes publicise well the clinical problems that may arise with drugs, but the mechanisms, investigation and prediction of drug reactions remain major problems, so that a monograph on 'Allergic Drug Reactions' is timely and welcome.

Dr Amos has produced a slender volume in which he couples immunology to drug reactions. After an introductory chapter describing the types of adverse drug reactions, the watch-dog committees and the elaborate screening procedures now employed by the pharmaceutical companies, there is a conventional account of the four known types of immunological injury. Eleven pages are devoted to a chapter on clinical manifestations and here the clinician will find a useful summary of the major problems but will be left at the end with a feeling of dissatisfaction. This is no fault of the author; it is simply a reflection of our limited knowledge of the exact mechanisms. Then follow chapters on drugs as antigens with particular emphasis on recent work on penicillin and its derivatives. Diagnosis and diagnostic procedures involving the patient—prick, scratch, intradermal, photo-patch and provocative tests are covered as are treatment of acute reactions and specific immunological treatment by hyposensitization. Four chapters detail the investigation of each of the types of immunological reaction and here the clinician will encounter the complexities of laboratory investigations, and perhaps wonder, at the end of it all, whether it would be useful to have a summary—in the form of a flow-chart—to guide him through the management and investigation of a patient with a drug reaction. Finally, there is a brief note on future prospects where mention is made of the genetic basis for certain immune responses and how this may one day allow identification of those most likely to mount adverse immune reactions. With the report from ICI that practolol was given seven years of rigorous tests, none of which showed any long-term side effects, more emphasis will presumably now be given to attempts to identify genetic or metabolic peculiarities that predispose to allergic drug reactions. It should not be long before this book is expanded into a companion for Meyler.

J.J.Cream

The Swollen Leg. Causes and Treatment. H. Daintree Johnson, & J. Pflug, (1975) London: Heinemann. Pp. 284, Figs. 79, Plate 1.

The treatment and management of the so-called 'varicose ulcer' is as badly taught as it is badly named. The causes, perpetuating factors and long-term control of patients with leg ulcers have tended to be relegated to the backwaters of medical practice. Yet this is an area of medicine that is intellectually demanding and emotionally satisfying. The opportunities of the application of logical principles to the factors responsible for the ulceration (and these are often complex) and the satisfaction of healing long-standing and painful ulcers and in rehabilitating the patient to the renewal of a full ambulant life should deserve more thought and application than is usually given to this common condition.

Perhaps because of lack of interest by others, perhaps because of logistics, many dermatologists find themselves with 'leg ulcer' clinics. Certainly by virtue of the frequency of complication induced by ill-advised overtreatment with topical agents and the interest they have in the microvascular circulation and in the mechanisms of vasculitis, dermatologists may do as well as anyone in treating these patients. They have a long tradition of doing so. They are the middlemen in a situation essentially surgical but with important medical associations.

This excellent book contains a close-reasoned account of the authors' views on the causation of venous ulceration, with chapters on lymphoedema and other aspects of the 'swollen leg'. The first part is concerned with a detailed account of the physics of the circulation of the lower limb and the mechanisms of oedema and lymphoedema. The authors are doubtless provocative in their insistence of the importance of shunting mechanisms in postulating a different conception of the post-thrombophlebitis state from that which is normally held. But they argue it well and base it on practical observations and abundant and sound physiological studies.

The second part deals with the resulting diseases, and the third part with treatment. It is these last chapters (9–17) which will most interest the dermatologist though he will certainly regret the short space given to the treatment of the venous ulcer. No mention is made of the high frequency of sensitization eczema in the treatment of these patients and the nutritional and social aspects are not discussed. But, to be fair, this is outside the brief of a work that is primarily concerned with the physics and mechanisms that lead to the oedematous leg.

A full appreciation of the earlier part demands some knowledge of physics but the reasoning and conclusion at each stage are admirably set out in clear summaries. The arguments put forward provide a logical approach to a common cause of morbidity that has seldom been paid the honour of a scientific study in such depth. All dermatologists who have the care of patients with ulcerated or swollen legs will be stimulated to look afresh at their patients after reading this book.

D.S.WILKINSON

Diagnostic Pratique des Maladies des Peaux Noires. A.A. DE TERWANGNE (1976) Published by the author, B.P. 40, Gisenyi, Rwanda, Africa. Pp. 202. (Copies may be purchased for U.S. $5, which includes postage. Cheques should be made out to 'Amis du Pere Damien', Account 70119, Banque Commerciale du Rwanda, P.O. Box 169, Gisenyi, Republic of Rwanda.)

The presence of heavy pigmentation, partially masking erythema, certainly increases the difficulty of establishing a morphological diagnosis, particularly for the dermatologist most of whose patients have lighter skins. Dr de Terwangne has attempted to simplify the process of reaching a diagnosis in the dark-skinned patient in a small soft-backed volume, which he intends mainly for the use of doctors who are not dermatologists, and of nurses. His approach to the problem is morphological and topographical; in a telegraphic style he briefly summarises and tabulates the diagnostic possibilities to be considered for each type of lesion in each region of the body. Each entry is followed by page references to one or both of Degos' well-known textbooks. A vast amount of information is provided, most of it equally applicable to all races, and the differential diagnoses are as well considered as the limited space allows. Recommendations for treatment are compressed into a line or even into a single word; R O is often wisely advised and would generally be preferred to the recommended cryotherapy for lichen nitidus.

The value of this work for those for whom it is intended is unquestionable and its compilation must have involved hundreds of hours of tedious labour. The value of the book to dermatologists in general would have been greatly enhanced if the author had drawn more on his personal experience, had confided himself to the diagnostic possibilities to be encountered in West Africa and had provided some incidence figures. Chagas disease appears next to anthrax; might it not have been preferable to omit the former to allow space for details of the incidence and epidemiology of anthrax in Rwanda ? It was right to include the rarities for as the author says in his introduction rare diseases are rare but they exist, but symbols could have been devised to indicate their geographical distribution, or in such conditions as Werner's syndrome, their universal rarity.

ARTHUR ROOK

Applied Medical Microbiology. J.G. COLLEE (1976) Oxford: Blackwell Scientific Publications. Pp. 121 + vi. Price £2.80.

There is a tendency amongst both cars and books for the small and eminently useful to grow bigger with each edition until it finally becomes too large to handle with ease. The value of this may lie in the challenge that it presents to someone to design or write a new small compact version which nevertheless retains all the essential features. Professor J.G.Collee has achieved just this with this book *Applied Medical Microbiology*. The essence of medical microbiology is distilled into 14 very short chapters; this is an excellent book for the tyro microbiologist or for those who wish to refresh their memories without ploughing through the minutiae of the subject. The text is enlivened with line drawings and cartoons. Other volumes in the same series cover general microbiology, microbial physiology, virology and food microbiology. At the low price of £2.80 this book should be in everyone's possession.

W.C.NOBLE

British Journal of Dermatology (1976) **95**, 575.

News and Notices

Psoriasis Research Award for 1977

The Department of Dermatology, Baylor College of Medicine, Houston, announces the Mr and Mrs J. N. Taub International Memorial Award for Psoriasis Research for 1977.

This annual award of $1000 was established in 1957 by Carol and Henry J. N. Taub in honour of their grandparents, Mr and Mrs J. N. Taub. Their establishment carried with it the hope that the award would be an incentive to develop a cure to alleviate one of the problems of mankind.

The award is designed for the individual or group whose investigative work in this field has been the most outstanding. Names of applicants for the award, together with supporting data, should be submitted for consideration by 1 December. Results of the research must have been presented before a dermatological medical society or published in a recognized medical journal.

The award's recipient will be determined by a committee of seven. If, in the committee's opinion, none of the applications reflect outstanding research, no award will be given for the year.

Address all communications to: John M. Knox, M.D., Professor and Chairman, Department of Dermatology, Baylor College of Medicine, Houston, Texas 77030, U.S.A.

Annual Hair Transplant Symposium and Workshop

This Symposium, organized by the Stough Dermatology and Cutaneous Surgery Clinic, Hot Springs, Arkansas, and co-sponsored by the American Society for Dermatologic Surgery, and the American Academy of Facial Plastic and Reconstructive Surgery, will be held on the 11 & 12 February 1977.

Further details may be obtained from Dr D. B. Stough III, Stough Dermatology and Cutaneous Surgery Clinic, P.A. Doctors Park, Hot Springs, Arkansas 71901, U.S.A.

Advanced Course in Dermatology. Simple Laboratory Techniques in the Diagnosis and Treatment of Skin Diseases: A Practical Course

This Annual Seminar is open to Consultants and Senior Registrars in Dermatology. It will also include clinical sessions. It will be held from 21 to 25 March 1977 at the Institute of Dermatology and St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ. Applications should be sent to the Dean before 31 December 1976.

Paediatric Dermatology Seminar Miami Beach, 24–27 February 1977

A 3-day Paediatric Dermatology Seminar will be held at the Konover Hotel, Miami Beach, 24–27 February 1977. This will be followed by a 1-week post seminar cruise to the Bahamas, etc. Dr R. Winkelmann will conduct daily lectures and discussions.

The course is designed to review the most recent advances in skin diseases of children. Speakers include: Dr R. K. Winkelmann, Dr R. Good, Dr S. Krugman, Dr H.Maibach, Dr E.Moynahan, Dr M.Viamonte and Dr G.Kahn.

Further details may be obtained from: Dr Guinter Kahn, 16800 NW 2 Ave., North Miami Beach, Florida 33169, U.S.A.

Dermatological training enrolment and accreditation

The Joint Committee for Higher Medical Training (JCHMT) published its second report in 1975.

This contains information about the training of specialists in many branches of medicine including dermatology. It has a supplement, which will periodically be brought up to date, listing the posts which have been recognized for training, subsequent to visits by teams appointed *ad hoc*. Copies of this report, price £1.50, may be obtained from the Secretariat of the JCHMT, the Royal College of Physicians, 11 St Andrew's Place, London NW1 4LE.

Earlier this year, the chairman of the JCHMT published a letter in the medical press (*British Medical Journal*, 1976, 17 Jan., p. 150) outlining the procedure to be adopted by trainees seeking enrolment with the JCHMT, and subsequent accreditation on the completion of their training period. A number of such requests have been made. At the AGM of the BAD in July, 1976, many members stated that they had heard neither of the 2nd report nor of the information on enrolment or accreditation. For this reason attention is drawn to these two important communications which affect not only trainees, but those responsible for them, and members of advisory committees for Senior Registrar and Consultant posts.

Dermatopathology Fellowship 1978–1979

The Dermatopathology Fellowship at New York University School of Medicine is designed to prepare dermatologists and pathologists for a career in dermatopathology within a medical centre environment. This will be accomplished by full participation in an active dermatopathology laboratory functioning within a department of clinical dermatology.

The Fellow will study microscopically the more than 150 skin specimens that are processed daily in the laboratory, attend clinical conferences in the Department of Dermatology and twice-weekly dermatopathology luncheon conferences where subjects of interest are discussed in depth, and be actively involved in planning and presenting the weekly dermatopathology conference.

The stipend is $15,000.00. Dr A.Bernard Ackerman, Director of Dermatopathology, is in charge of this program and serves as tutor and supervisor. Address inquiries to: A.Bernard Ackerman, M.D., 562 First Avenue, New York City 10016, U.S.A., 212-679-3200 ext. 2766.

The Second Regional Conference of Dermatology (Asian-Australasian)

This conference is organized by the Dermatological society of Thailand, the Ministry of Public Health and SEAMEO-Tropical Medicine and Public Health Project, and will be held in Bangkok, Thailand, from 17 to 20 January 1977. The most important aim is to assemble together those who are working in various fields of dermatology in countries which are neighbours, but delegates and observers from all other parts of the world are also welcome. The themes for discussion include: (1) venereal diseases; (2) leprosy; (3) mycology; (4) industrial dermatoses; (5) recent advances in dermatology; (6) free communications. Distinguished speakers from overseas are being invited to participate. For further information and application forms, please contact the Executive Secretary General of the Organizing Committee, Dr Tongnan Vibhatavavija, Institute of Dermatology, 42017 Rajavidhi Road, Bangkok 4, Thailand. Tel. 2828898.

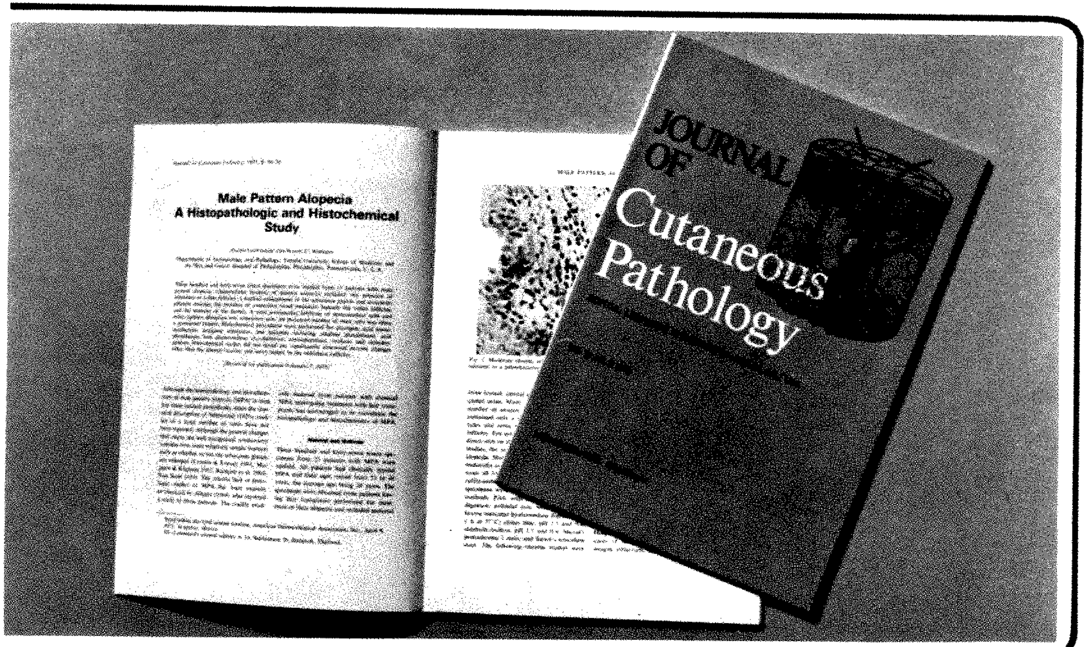
APPOINTMENT

University of Glasgow

Dr J.C.Gentles has been elected 'Titular Professor in Dermatology (Medical Mycology)' from October 1976.

An Honorary LL.D. has been awarded to Professor Hermann Pinkus.

Dermatology



Journal of
Cutaneous Pathology

Editor: Leopoldo F. Montes, Birmingham, Alabama

Journal of Cutaneous Pathology publishes manuscripts of the highest scientific quality in the field of cutaneous pathology. It aims at promoting and maintaining communication between clinicians and research workers. Cutaneous pathology will be interpreted to mean diseases of the epidermis, dermis and cutaneous appendages.
Original scientific articles on diagnostic and experimental cutaneous pathology are invited. Clinico-pathological studies will also be considered for publication.

As a special offer to new subscribers to JOURNAL OF CUTANEOUS PATHOLOGY we can offer you volumes 1 and 2 (1974 & 1975) FREE as long as the stock lasts so you can have the journal right from the beginning.

MUNKSGAARD · COPENHAGEN

Order Form to be sent in an envelope to:

MUNKSGAARD
35,Nørre Søgade
DK-1370 Copenhagen K.
Denmark

JOURNAL OF CUTANEOUS PATHOLOGY

☐ Please enter my subscription to start with volume 3, 1976. Subscription price D.Kr. 320.00 plus postage D.Kr. 14.00 (US$ 56.50 incl.postage).

☐ Please send me volumes 1 and 2 FREE (special offer to new subscribers only).

☐ I enclose .... in payment.

Name: ....

Address: ....

....

....

TEXTBOOK OF DERMATOLOGY

Available again as a reprint of the Second Edition

Edited by Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. *Second Edition*, 1972 (*Reprinted* 1975). 2236 pages, 1100 illustrations. Two volumes. £48.00

'The appearance of the second edition of this book comparatively soon after the first testifies the success with which it has fulfilled a need.... these two volumes are essential to the dermatologist; the trained dermatologist will need them for frequent reference, while for the trainee they should be the sheet-anchor of systematic study.' *British Journal of Dermatology*

'In their second edition the authors have expanded on the knowledge previously presented and have added several quite useful and appropriate sections. The textbook will continue to be a superb reference book which should be available to all dermatologists; it is admirable as a comprehensive reference, detailed, and well-written survey of the field of dermatology.' *The Journal of Investigative Dermatology*

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

HISTOPATHOLOGY OF THE SKIN

Walter F. Lever M.D. and Gundula Schaumburg-Lever M.D. *Fifth Edition*, 1975. 816 pages, 436 illustrations (8 colour). Lippincott, £36.40

In the eight years that have passed since publication of the fourth edition so much new information has become available on nearly every major dermatosis that the book had to be entirely rewritten. The greatest changes were required in the description of the lymphomas because they have been reclassified in recent years. Many changes have been made also in the description of the bullous dermatoses and of the metabolic diseases, especially of amyloidosis, colloid milium, hyalinosis cutis et mucosae, and porphyria. Also the division of malignant melanoma in situ into two types with different prognosis has been given due consideration.

More than 20 diseases not mentioned in the fourth edition are described in this edition. Some of them are newly recognized entities, such as disseminated superficial actinic porokeratosis, perforating folliculitis, reactive perforating collagenosis, angiolymphoid hyperplasia with eosinophilia, infantile digital fibromatosis, trichilemmoma, and malignant eccrine poroma. Also several previously known diseases because of their increased interest for the dermatologist have been incorporated, among them regional enteritis (Crohn's disease), Tangier disease, lipogranulomatosis (Farber's disease), and generalized eruptive histiocytoma.

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Aldenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldly names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	seconds(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm³	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, **81**, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: Beare, J.M. & Wilson Jones, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066, Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 95, Number 5, November 1976

CONTENTS

Clinical and Laboratory Investigations

PAGE

The Sunburn Cell in Mouse Skin: Preliminary Quantitative Studies on its Production 459
A.WOODCOCK AND I.A.MAGNUS

Thymus-dependent (T) Lymphocyte Deficiency in Progressive Systemic Sclerosis 469
P.HUGHES, SHIRLEY HOLT, N.R.ROWELL AND JANIS DODD

Macrophage Inhibitor Factor (MIF) in Cutaneous Lymphoproliferative Diseases 475
PABLO UMBERT, RICHARD W.BELCHER AND R.K.WINKELMANN

Lymphokines (MIF) in the Serum of Patients with Sarcoidosis and Cutaneous Granuloma Annulare 481
PABLO UMBERT, RICHARD W.BELCHER AND R.K.WINKELMANN

Granuloma Annulare: Direct Immunofluorescence Study 487
PABLO UMBERT AND R.K.WINKELMANN

Hypersensitivity to Bacteria in Eczema. IV concluded. Cytotoxic Effect of Antibacterial Antibody on Skin Cells Acquiring Bacterial Antigens 493
W.E.PARISH, ESTHER WELBOURN AND R.H.CHAMPION

The Ultrastructure of the Human Epidermis Following Ultracentrifugation 507
G.H.FINDLAY AND N.v.d.W.LIEBENBERG

The Epidermis in Thyroid Disease 513
P.J.A.HOLT, J.LAZARUS AND R.MARKS

Plasma Volume, Intravascular Albumin and its Transcapillary Escape Rate in Patients with Extensive Skin Disease 519
HANS-HENRIK PARVING, ANNE-MARIE WORM ANS NIELS ROSSING

Physical Training and Connective Tissues in Young Mice. Biochemistry of Skin 525
A.KIISKINEN AND E.HEIKKINEN

Localized Pemphigoid 531
JOHN R.PERSON, ROY S.ROGERS III AND HAROLD O.PERRY

Bullous Pemphigoid and Psoriasis: Does Subclinical Bullous Pemphigoid Exist? 535
JOHN R.PERSON AND ROY S.ROGERS III

Bullous Dermatosis of Chronic Renal Failure 541
KALMAN KECZKES AND MALCOLM FARR

Case Reports

Caustic Ulcers Caused by Cement 547
MATTI HANNUKSELA, RAIMO SUHONEN AND JAAKKO KARVONEN

Metastatic Crohn's Disease 559
D.I.MCCALLUM AND W.M.GRAY

Paroxysmal Itching in Multiple Sclerosis 555
P.O.OSTERMAN

Generalized Warts and Immune Deficiency 559
T.M.S.REID, N.G.FRASER AND I.R.KERNOHAN

Brief Communications

Improved Technique for Epidermal Cell Cycle Analysis 565
F.W.BAUER AND R.M.de GROOD

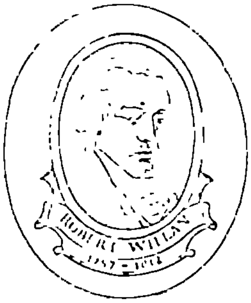
Effect of Hydrocortisone Butyrate on Collagen Synthesis 566
HEIKKI SAARNI AND VÄINÖ K.HOPSU-HAVU

PUVA-induced Bullous Pemphigoid 568
KRISTIAN THOMSEN AND HENNING SCHMIDT

Theses and Book Reviews 571

News and Notices 575

Volume 95, Number 6, December 1976



British Journal of Dermatology

Published for the
British Association of Dermatologists
by Blackwell Scientific Publications
Oxford London Edinburgh Melbourne

ISSN 0007 0963

British Journal of Dermatology

EDITOR

DR R. H. CHAMPION
Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ

EDITORIAL ADVISORY BOARD

DR C.N.D.CRUICKSHANK, Birmingham
Experimental pathology

PROF. F.J.EBLING, Sheffield
Zoology

DR J.C.GENTLES, Glasgow
Mycology

MR J.W.HADGRAFT, Cambridge
Pharmacy

PROF. HERMANN LEHMANN, Cambridge
Biochemistry

PROF. I.A.MAGNUS, London
Photobiology

PROF. MARY MARPLES, Woodstock
Microbiology

DR W.E.PARISH, Sharnbrook
Experimental pathology

DR A.C.STEVENSON, Oxford
Genetics

PROF. J.L.TURK, London
Immunology

MR G.S.WALTON, Liverpool
Comparative dermatology

ASSISTANT EDITORS

DR P.W.M.COPEMAN, London

DR RONALD MARKS, Cardiff

DR P.D.MIER, Nijmegen

DR J.N.S.MITCHELL, Royal Air Force

DR W.C.NOBLE, London

DR R.S.WELLS, London

EDITOR OF SOCIETY PROCEEDINGS

DR J.N.S.MITCHELL,
40 Meadow Way, Letchworth, Herts.

British Journal of Dermatology is published monthly. Subscription price £21.00 U.K. (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed in Great Britain at the Alden Press, Oxford

'OUTLOOK IN PSORIASIS'

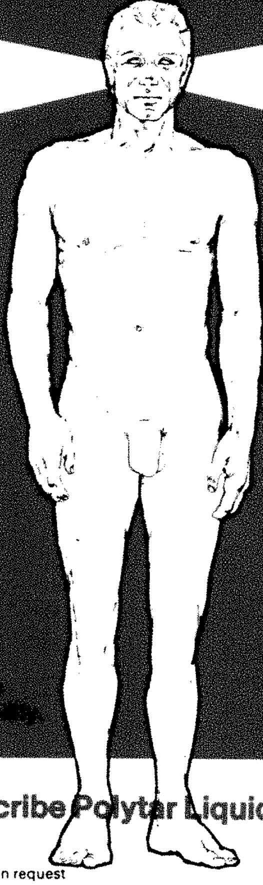
'The time-honoured applications of tar or dithranol and exposure to ultra-violet light remain the basis for local treatment'

Editorial, Brit. med. J. 1974,4,365

Polytar Liquid Psoriasis of the scalp

In a recent teaching hospital study, Polytar Liquid was compared with a standard psoriasis formulation in [illegible] patients with psoriasis of the scalp. Polytar Liquid proved superior in the removal of scale and the relief of [illegible]. Patient acceptability was excellent.

Polytar Liquid combines effective control with patient acceptability



Polytar Emollient Psoriasis of the body

[illegible]

[illegible]

[illegible]

For psoriasis – prescribe Polytar Liquid and Polytar Emollient

Full information on request

Stiefel Laboratories (UK) Limited, Slough, SL1 4OQ

IMPORTANT NEW PRODUCT FOR PSORIASIS

Dithranol
olan

Much easier to apply, far easier to remove, and actually more effective.

DITHROLAN is quite simply the most convenient and effective form of dithranol in the treatment of psoriasis.

A careful and painstaking study carried out over the last 5 years at the Beaumont Hospital, Lancaster, demonstrated that a newly developed formulation of dithranol, now introduced as DITHROLAN, was not only very much easier to use than traditional Dithranol Paste, but was actually more effective[1,2].

DITHROLAN adheres easily to the skin and can be applied quickly and conveniently. Previous applications of DITHROLAN can be removed simply by bathing – obviating the need for oil. As a result, patients actually carry out their treatment.

Dermatologists will appreciate DITHROLAN because of the considerable saving in treatment effort and time, and because of the excellent results obtained.

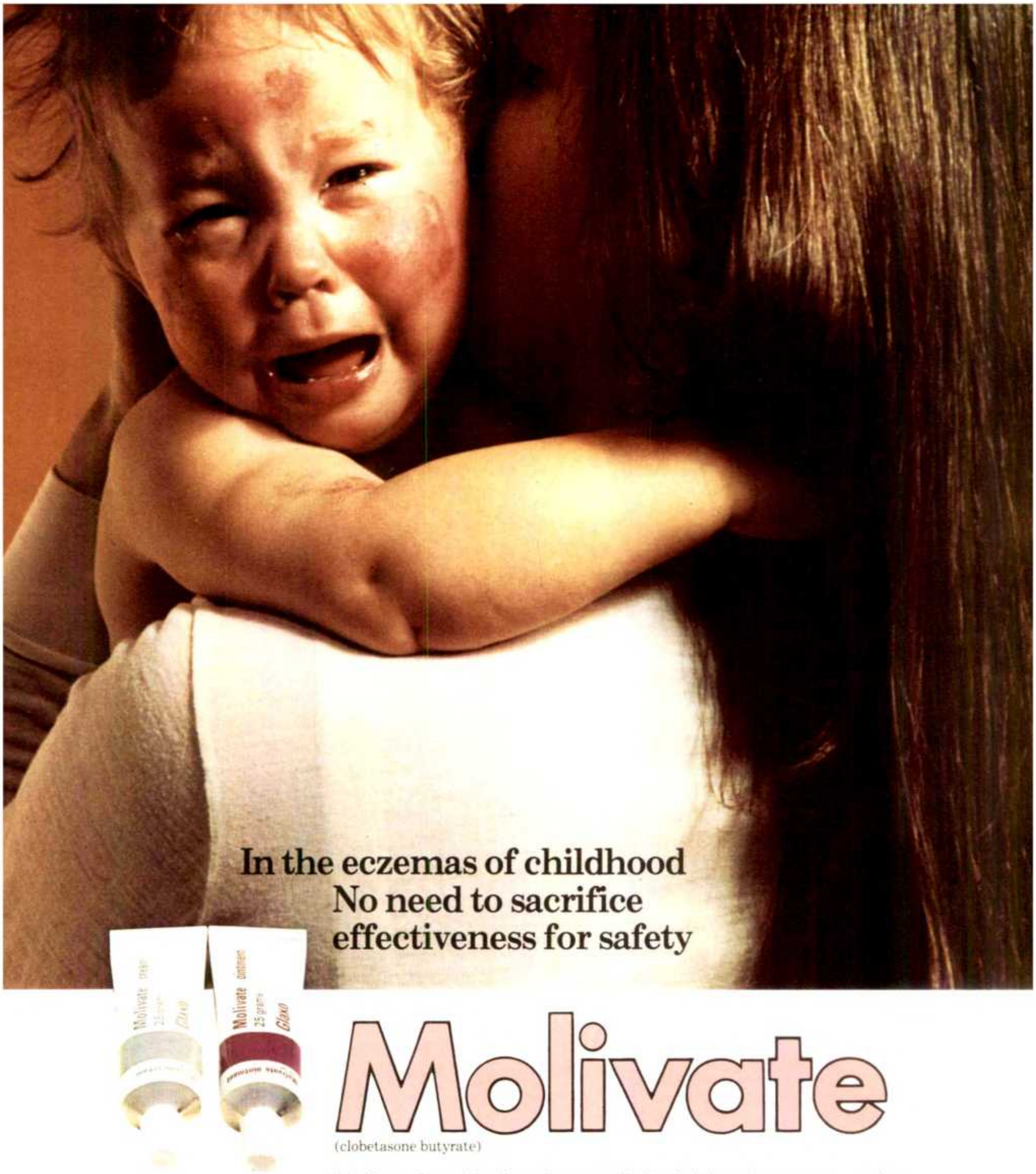
Presentation: DITHROLAN – containing 0·5% Dithranol B.P, 0·5% Salicylic Acid B.P. 90 gram amber glass jar. PL 0173/0024

References: 1. Brit. J. Derm. **93,** *205, 1975. 2. Dermatology Digest* **Feb,** *20, 1976.*

Further information and literature available from:

Dermal Laboratories Limited,
Tatmore Place, Gosmore, Hitchin,
Herts, SG4 7QR, England

Dithrolan



Molivate

(clobetasone butyrate)

Molivate is an ideal corticosteroid for childhood eczemas – such as atopic eczema – because it has been shown to be significantly more effective than hydrocortisone, yet to have a *wide margin of safety*.*

Cream – particularly suitable for the wet and weeping lesion.
Ointment – for the dry lichenified lesion.

British Medical Journal* (1975) **3, 626

Molivate is available in 25 and 100 gram tubes

Glaxo

Full prescribing information on Molivate (trade mark) is available from Glaxo Laboratories Ltd, Greenford, Middlesex UB6 0HE

CHARLES C THOMAS • PUBLISHER

THE ULTRASTRUCTURE OF COLLAGEN: Its Relation to the Healing of Wounds and to the Management of Hypertrophic Scar *edited by* **J. J. Longacre.** *(35 Contributors)* Scanning and transmission electron microscopic studies are presented along with observations on the influence of age on wound healing and the role of environmental factors on connective tissue cell metabolism. The biochemical and histological advances in hypertrophic scar management are also presented. A final chapter discusses the use and abuse of scar tissue in light of recent biophysical and biochemical studies. *'76, 537 pp. (6 3/4 x 9 3/4), 537 il., 28 tables, $41.50*

BEHAVIORAL SCIENCE IN CLINICAL MEDICINE *by* **Stewart Wolf,** *Univ. of Texas Medical Branch, Galveston, and* **Helen Goodell,** *Cornell-New York Hospital Medical Center, New York, New York.* Health and well-being depend not only on man's capacity to adapt to the tangible environment, but also to prevailing attitudes and values in his society and to his own goals and aspirations. The author discusses behavioral science in the context of human ecology, man's needs and expectations, the significance of the individual in medical education and practice, and values and fulfillment. *'76, 256 pp., 22 il., $14.50*

PURCHASING FOR THE HEALTH CARE FACILITY *by* **John H. Holmgren,** *St. Joseph Medical Center, Wichita, Kansas.* There is a need today for an understanding of health care facility purchasing. This book covers the major areas of purchasing from stores and inventory to negotiation and planning. Included are ways of reducing costs, vendor relations, the departmental manual, traffic and transportation, health care purchasing as a profession, and future trends in health care material management. *'75, 288 pp., 18 il., 29 tables, $18.50*

THE DIAGNOSIS AND TREATMENT OF FUNGAL INFECTIONS: A Symposium *compiled and edited by* **Harry M. Robinson, Jr.,** *Univ. of Maryland, Baltimore, Maryland. (38 Contributors)* The experienced dermatologist and medical mycologist as well as the internist will find this text to be of value. The contents cover recent developments in microbiology, laboratory diagnosis, histopathology, immunology and recent experimental work. Therapeutic procedures described represent the latest approved methods. *'74, 580 pp., 128 il. (18 in color), 45 tables, $29.50*

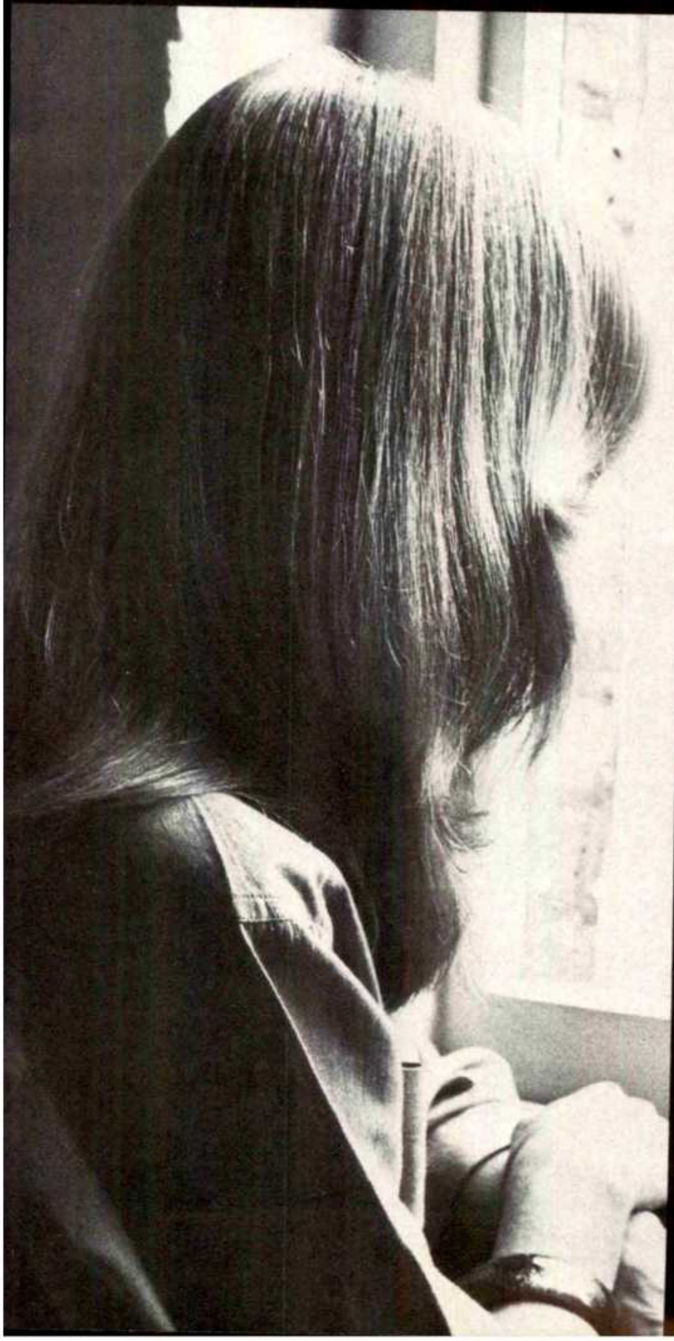
SCLERODERMA: Progressive Systemic Sclerosis *by* **Alfred J. Barnett,** *Alfred Hospital, Melbourne, Australia.* In addition to extensive observations at the clinical level, important research work is reviewed on the more fundamental aspects including disturbance in connective tissue metabolism and immunology. Chapters deal with pathology, vascular disturbance, connective tissue disturbance, immunological disturbance, clinical manifestations and course, affection of particular systems, diagnostic problems and treatment. *'74, 270 pp. (6 3/4 x 9 3/4), 84 il. (9 in color), 19 tables, $24.75*

NAIL DISEASES In Internal Medicine *by* **Pietro de Nicola,** *Univ. of Pavia, Pavia, Italy,* **Mario Morsiani** *and* **Giorgio Zavagli,** *both of the Univ. of Ferrara, Ferrara, Italy.* The principal part of the book is concerned with the clinical aspects of nail disorders, including the most relevant nail symptoms. A clear description is presented of all nail symptoms, most of which are not well known and deserve consideration in clinical practice. Original illustrations, most of which are color plates, are particularly helpful in the description of nail symptoms and disorders. *'74, 128 pp., 18 il. (8 in color), $10.50*

Orders with remittance sent by air, postpaid

301-327 East Lawrence Avenue • Springfield • Illinois • 62717 • USA

She's been to the chemist
she's been to her doctor
now she's come to you



In acne "... not all patients respond satisfactorily [to tetracycline] and there is a need for more effective or alternative therapy."

SEPTRIN* "... has been clinically effective in patients who did not respond to long-term tetracycline."

Brit J Derm, (1971), **84**, 366.

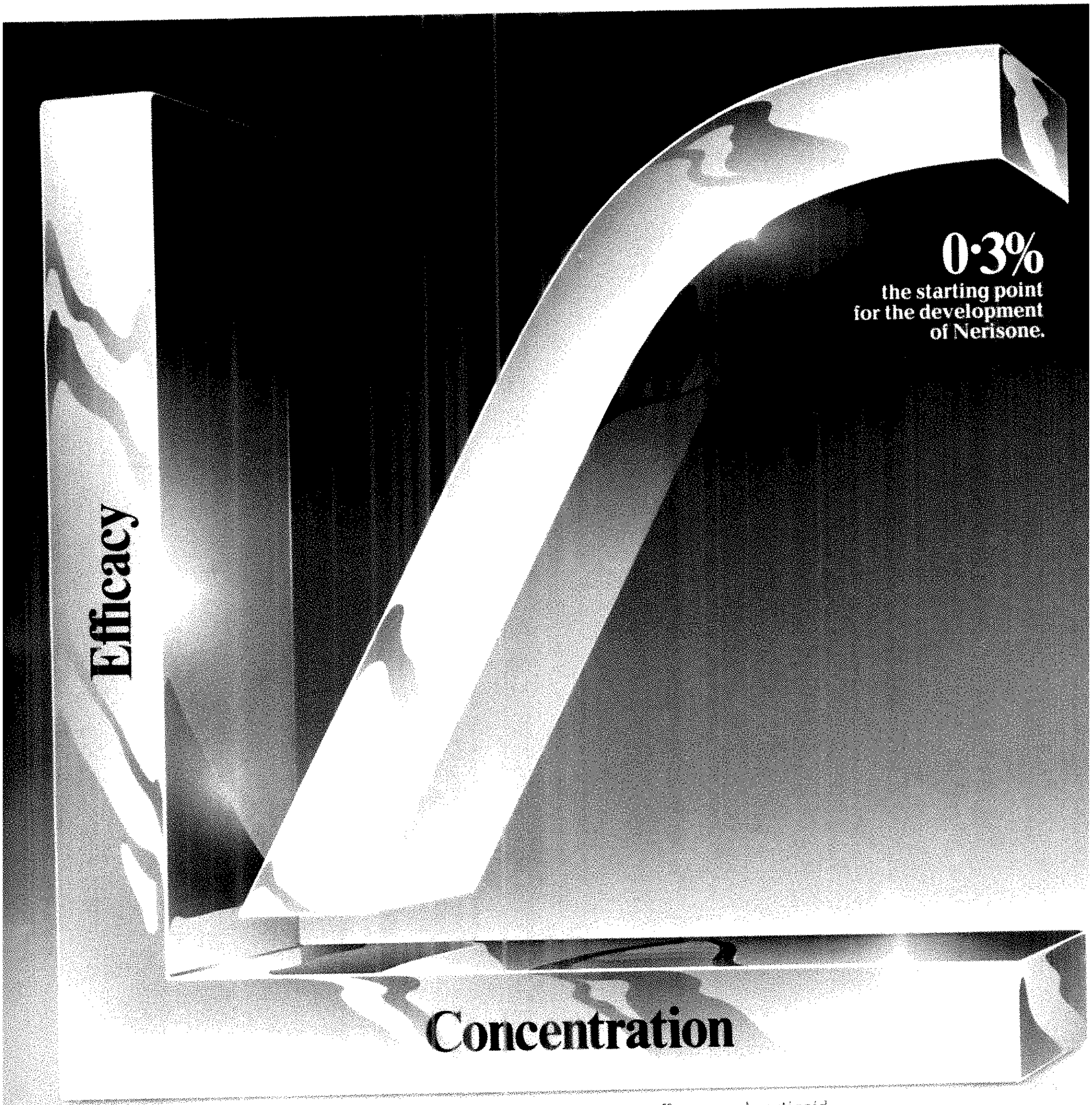
SEPTRIN FOR ACNE

Wellcome

Septrin contains trimethoprim and sulphamethoxazole. Full prescribing information is available on request.
Wellcome Medical Division
The Wellcome Foundation Ltd.
Berkhamsted, Herts

*Trade Mark

SCHERING
A NEW APPROACH



Graph showing the relationship between efficacy and corticoid concentration with diflucortolone valerate.

Product Information
Presentations Cream (oil-in-water emulsion), oily cream (water-in-oil emulsion) and ointment (anhydrous base). 1 gram of each Nerisone presentation contains 1 mg (0.1%) diflucortolone valerate. *
Uses Corticoid-responsive dermatoses in the absence of infection.
Dosage and administration Initially 2-3 applications daily, according to the severity of the condition. For maintenance one application daily.

Nerisone is available in three bases. The base chosen for an individual case should depend on the physical characteristics of the lesion. For example, in very dry and chronic skin conditions, the occlusive effect of the Nerisone ointment base may often aid the healing process.
Contra-indications, warnings, etc. *Contra-indications* Cutaneous syphilis and tuberculosis in the treatment area, smallpox, chicken-pox and vaccinia. Nerisone is not suitable for the treatment of ophthalmic conditions.

Warnings/Side effects Topical administration of corticoste
to pregnant animals can cause abnormalities of foetal
development. The relevance of this finding to human bein
not been established; however, topical steroids should not
used extensively during the first trimester of pregnancy, i.
large amounts or for prolonged periods.
As with all topical corticoids, both local and systemic side
can occur in certain circumstances.
Prolonged exposure (e.g. for more than 4 weeks) may lead

BRING TO SKIN THERAPY

Neither too strong nor too weak, Nerisone has been specifically developed for everyday cases of eczema and dermatitis since these do not need superfluously strong products nor will they respond quickly to the limited efficacy of hydrocortisone products.

Nerisone-developed from the most effective concentration

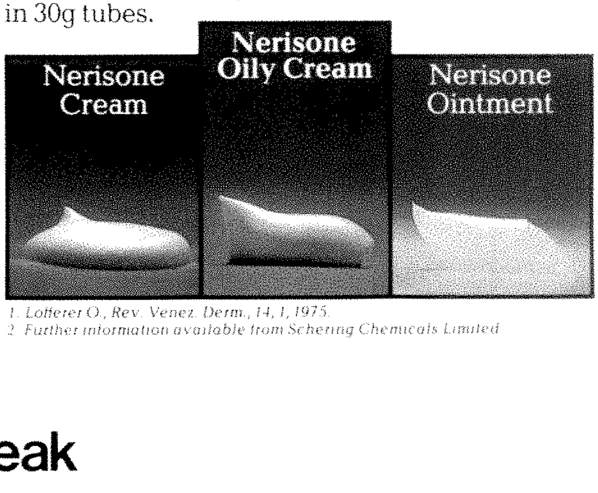
In a widely used dermatological test,[1] diflucortolone valerate at a concentration of 0·3% produced more positive results than probably the most potent topical corticoid to date; the real development was to reduce the concentration progressively to 0·1% to arrive at Nerisone which thus has a desired balance between efficacy and safety.

Nerisone-controlled efficacy balanced with excellent tolerance

World-wide clinical trials[2] involving 6,500 patients have shown Nerisone to be over 90% effective in the treatment of eczema and dermatitis while maintaining excellent local and systemic tolerance.

Nerisone-a new base, one tube size

In addition to the conventional cream and ointment bases, new Nerisone is also available in a special OILY CREAM base which combines the softening and occlusive qualities of an ointment with the cosmetic acceptability of a cream. To simplify prescribing, Nerisone is only available in 30g tubes.



---

[1] *Lofferer O., Rev. Venez. Derm., 14, 1, 1975.*

[2] *Further information available from Schering Chemicals Limited*

Neither too strong nor too weak

NEW Nerisone

balanced to avoid the extremes of corticoid therapy

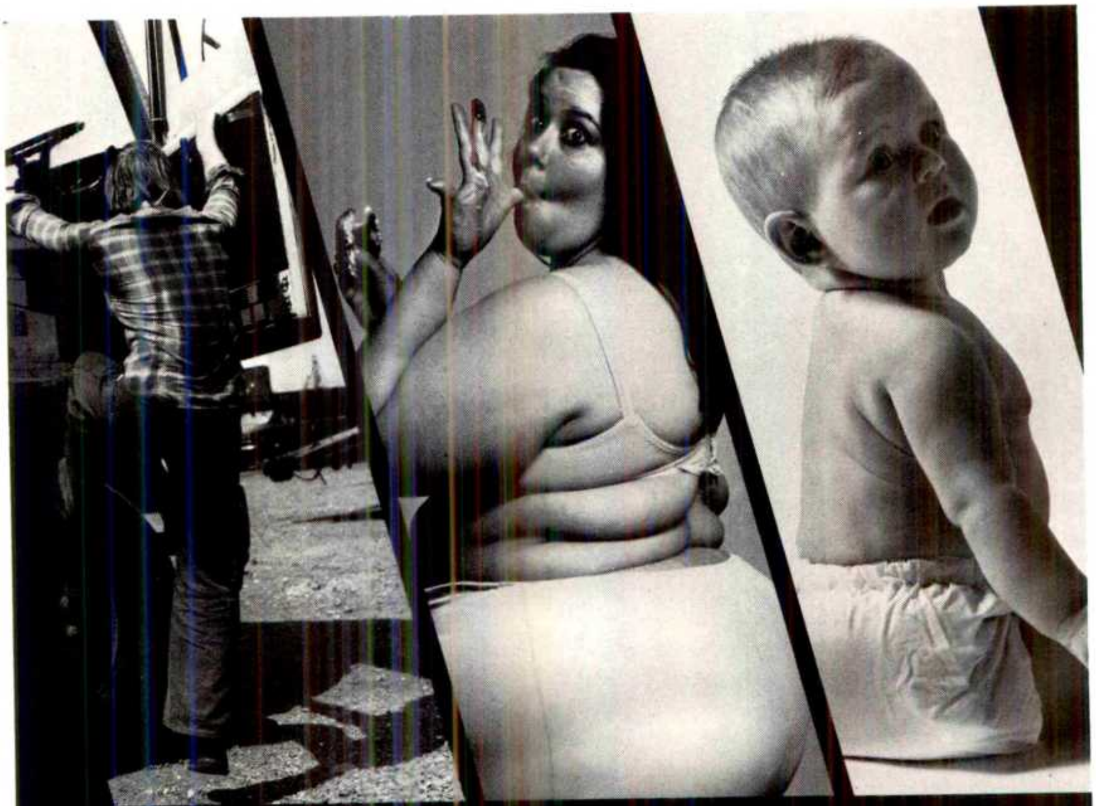
Schering Chemicals Ltd., Pharmaceutical Division,
Burgess Hill, West Sussex RH15 9NE.

local effects such as atrophy of the skin, striae, telangiectasia or acneform changes, espically when the ointment base is used, or Nerisone is applied under occlusion.

When large areas are treated (again especially with the ointment base or under occlusion), natural steroid production may be depressed. The following precautions should therefor be observed:

1) Areas larger than 10% of the body surface should not be treated for more than four weeks.
2) Occlusive dressings should not be left on for more than 24 hours. They may be used repeatedly, but it should be noted that the risk of systemic effects is thereby increased.
3) If secondary infection occurs during treatment, the use of occlusive dressings should be stopped until the infection has been eliminated.
4) Children up to the age of 4 years should not be treated with Nerisone for longer than three weeks–particularly on skin areas covered by napkins.

Pharmaceutical precautions Store in cool, dry conditions.
Legal category TSA
Package qualities 30g tubes
N.H.S. Price 30g: £1.49
Further information Available on request
Product licence numbers Nerisone Cream 0053/0075 Nerisone Oily Cream 0053/0073 Nerisone Ointment 0053/007



ano-genital pruritus, intertrigo, nappy rash – special skin problems that need the special properties of Timodine.

The special problems of skin-fold dermatoses are those caused by warmth and moisture: maceration and chafing, leading to secondary bacterial and fungal infection, with severe inflammation and pruritus.

Timodine offers a comprehensive formula to counter these problems: nystatin and benzalkonium chloride for antibacterial and anti-fungal effect, hydrocortisone to reduce inflammation and pruritus without the hazards of fluorinated steroids, and dimethicone 350 to protect the skin from moisture.

Timodine

every time

Timodine contains (w/w) Dimethicone 350 B.P.C. 10% Nystatin B.P. 100,000 i.u./g, Benzalkonium Chloride Solution B.P. 0.2%, Hydrocortisone B.P. 0.5%. Tubes of 30g. PL 1839/0001.

Full prescribing information is available.
Lloyd-Hamol Ltd. A member of Reckitt & Colman
Pharmaceutical Division, Hull, HU8 7DS.
Timodine is a registered trade mark.

PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
PSORIASIS
the modern basis of treatment

'... to restrict topical steroid therapy to short periods for getting the disease under control and then to change to tar preparations which may prevent rebound exacerbations.'
Practitioner, 1970, **204**, 625.

New Alphosyl HC cream
for short-term control
The unique descaling, healing and anti-inflammatory properties of Alphosyl, plus the added anti-inflammatory action of hydrocortisone to get the disease swiftly under control.

Alphosyl cream
lotion and shampoo for long-term maintenance
'... it (Alphosyl) does not have the disadvantage of staining, burning and discolouration that occur with other topical preparations.' Br.med.J., 1967, **4**, 535.

Alphosyl
a whole range of treatment for psoriasis

Alphosyl cream, lotion and shampoo contain a highly refined extract of coal tar and allantoin. Alphosyl HC in addition contains 0.5% hydrocortisone. Please send to us for samples, clinical information and Data Sheets.

Stafford-Miller The Professional Relations Division, Stafford-Miller Limited, Hatfield, Herts.

Churchill Livingstone

MECHANISMS OF TOPICAL CORTICOSTEROID ACTIVITY

A Glaxo Symposium

Edited by Lyn Wilson and R. Marks 160 pages illustrated £9·00

Traditionally Glaxo Symposia have had three aims: to provide a forum for the presentation and discussion of new work, to bring together workers in different disciplines who may not otherwise have an opportunity to exchange views on a topic of common interest, and to provide a publication that is a useful contribution to the literature.

Steroids have been used locally, by several specialities for almost a quarter of a century. Ophthalmologists, dermatologists and gastroenterologists, for example, have long recognised the advantage of applying an active glucocorticoid directly to the target organ as a means of avoiding the unwanted generalised effects consequent upon systemic administration. This book includes new work on the effect of glucocorticoids on blood vessels, cell synthesis and melanin pigmentation as well as epidermal thinning, wound healing and the pharmacological mediators of inflammation. There is also a comprehensive review of structure activity relationships, and the end paper relates the particular activities described in the intervening chapters to the general theory of corticosteroid activity.

Churchill Livingstone 23 Ravelston Terrace Edinburgh EH4 3TL

TEXTBOOK OF DERMATOLOGY

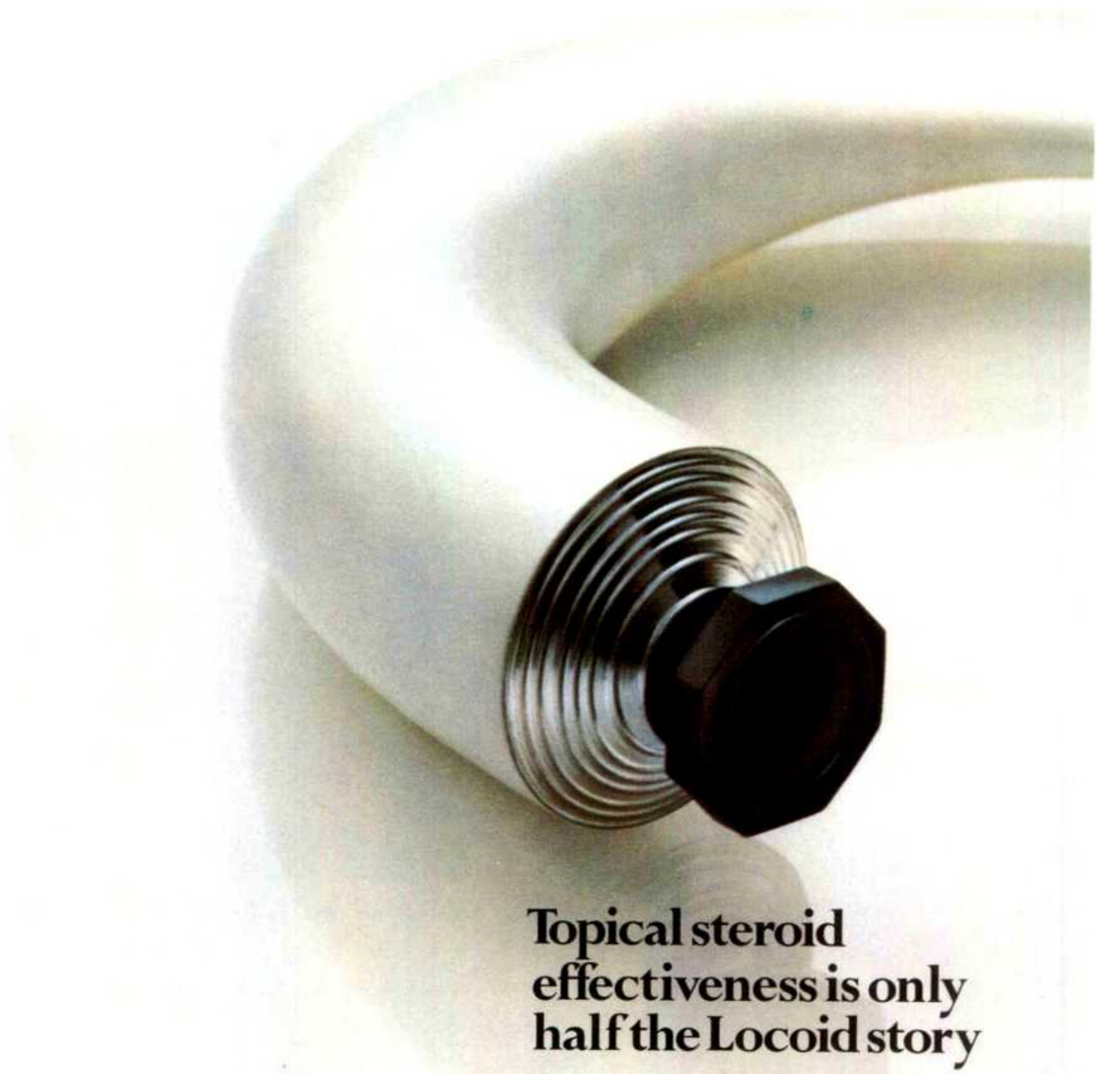
Available again as a reprint of the Second Edition

Edited by Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and F.J.G. Ebling D.Sc. Ph.D. *Second Edition*, 1972 (*Reprinted* 1975). 2236 pages, 1100 illustrations. Two volumes. £48.00

'The appearance of the second edition of this book comparatively soon after the first testifies the success with which it has fulfilled a need. . . . these two volumes are essential to the dermatologist; the trained dermatologist will need them for frequent reference, while for the trainee they should be the sheet-anchor of systematic study.' *British Journal of Dermatology*

'In their second edition the authors have expanded on the knowledge previously presented and have added several quite useful and appropriate sections. The textbook will continue to be a superb reference book which should be available to all dermatologists; it is admirable as a comprehensive reference, detailed, and well-written survey of the field of dermatology.' *The Journal of Investigative Dermatology*

Blackwell Scientific Publications
Oxford London Edinburgh Melbourne



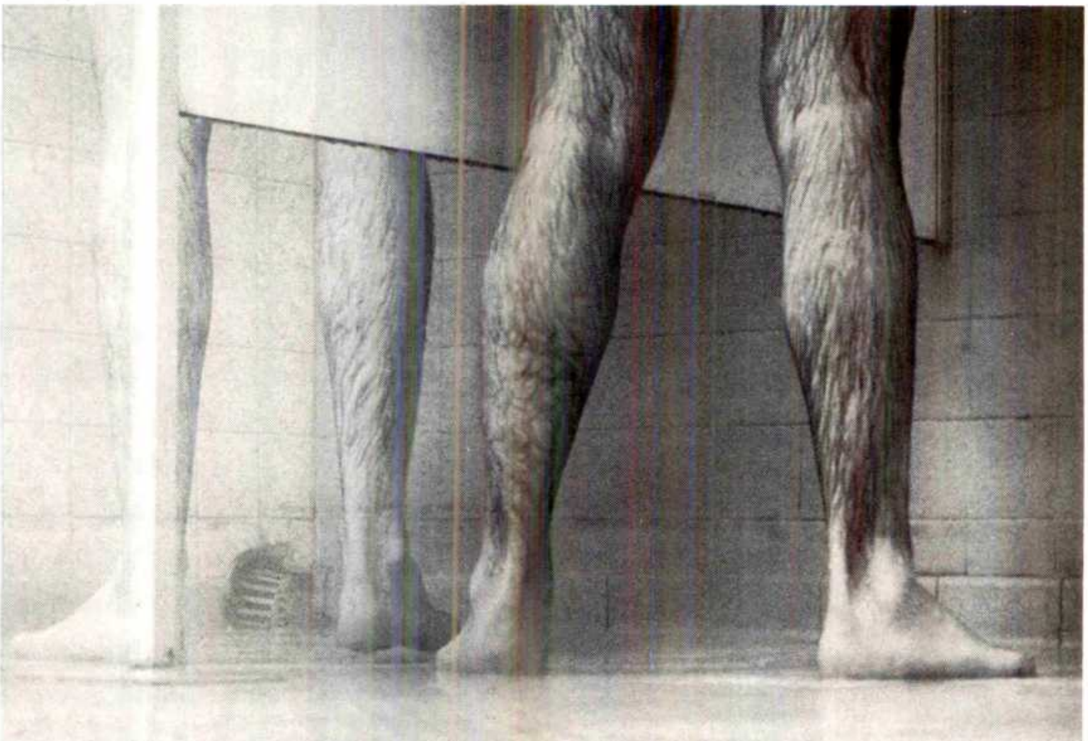
Topical steroid effectiveness is only half the Locoid story

...the other half is the relative freedom from topical side effects which are often encountered with the fluorinated steroids. Locoid offers the same level of success as these potent preparations–without fluorination.

locoid

the non-fluorinated way to topical steroid success

Locoid is a trade mark for hydrocortisone 17-butyrate.
Full information available on request:
Brocades (Great Britain) Ltd.,
Brocades House, West Byfleet, Surrey, KT14 6RA.
Telephone: Byfleet 45536.



The new solution for
fungal skin infections

New Canesten Solution

Canesten®
clotrimazole
broad-spectrum antifungal

More and more doctors are finding Canesten a highly effective and trouble-free treatment for all the fungal infections commonly seen in hospital practice.

In Gynaecology:
6-day Canesten
the only effective 6-day treatment for candida and mixed vaginal infections.

In Dermatology:
broad-spectrum Canesten
now the most widely prescribed treatment for fungal skin infections.

As effective as Canesten cream in treating a wide range of fungal skin infections

Especially suitable for applying to sensitive, moist or hairy areas

Also suitable for fungal infections of the outer ear (otitis externa)

Both the cream and the new solution are easy to apply, non-greasy, non-staining and odourless

Bayer

Further information, including data sheet, is available from
Bayer UK Limited, Pharmaceutical Division,
Haywards Heath, West Sussex RH16 1TP
Telephone: Haywards Heath (0444) 57911
® Registered Trade Mark of Bayer Germany

British Journal of Dermatology (1976) 95, 577.

Clinical and Laboratory Investigations

The cell cycle in psoriasis: a reappraisal

SEYMOUR GELFANT

Departments of Dermatology and Cell and Molecular Biology, Medical College of Georgia, Augusta, Georgia 30902, U.S.A.

Accepted for publication 7 June 1976

SUMMARY

The current belief that the clinical manifestations of psoriasis (excessive scaling) are due to a twelve-fold speeding up or shortening of the cell division cycle time of the germinative cells in psoriatic epidermis (from 457 to 37·5 h) is shown to be incorrect. A new concept is introduced—that the germinative layer in human epidermis is composed of not one, but three separate and distinct populations of epidermal cells. First, there are cycling cells which are actively moving through the cell cycle. Then there are two categories of non-cycling cells (blocked in the G_1 or the G_2 periods of the cell cycle) which are capable of moving into the proliferative pool upon specific stimulation. Thus, increased epidermal cell proliferation in active lesions of psoriasis would be brought about mainly by a recruitment or a release of the two categories of non-cycling cells. The idea that germinative epidermal cells are primarily non-cycling, leads to the suggestion of focusing attention on non-cycling cells (rather than on cycling cells) for the control and treatment of psoriasis. It might be worthwhile considering treating psoriatic patients during periods of clinical remission—with factors to keep the germinative cells in the non-cycling state—rather than during psoriatic flare up—with cancer chemotherapy drugs.

Psoriasis has been described as a disease of epidermal hyperplasia which is manifested clinically by excessive production of scale (Weinstein & Frost, 1968). Psoriatic epidermis is much thicker than normal and contains a larger proliferative or germinative cell population—located in the lower few layers of epidermis. The upward movement, or transit, of germinative cells through the thicker psoriatic epidermis is much faster, i.e. 2 vs 14 days in normal epidermis. The rapid transit time of cells moving up through the multi-layered epidermis and stratum corneum accounts for the excess of scaling in psoriasis. The force that drives this upward movement of cells comes from accelerated proliferation of germinative cells—some of which move up or push their neighbours up and out of the basal layer of epidermis.

Accelerated proliferation of germinative cells in psoriatic epidermis is thought to be due to a speeding up or shortening of the cell division cycle time, i.e. from 457 h (19 days) in normal epidermis to 37·5 h (1½ days) in psoriatic epidermis (Weinstein, Velasco & Frost, 1971). (The 37·5 h cell cycle time has been used to classify the psoriatic epidermal cell as one of the most rapidly proliferating cells in the body [Weinstein & Frost, 1971].) The values of 457 h for normal epidermis and 37·5 h for psoriatic epidermis germinative cell cycle times were introduced by Weinstein and co-workers (1968–71, for refs see Table 1). These two cell cycle time values serve as their explanation for the clinically elevated scaling seen in psoriasis and also for their rationale for treating psoriasis with cancer chemotherapy drugs such as methotrexate (Weinstein, 1971a; Weinstein & McCullagh, 1976). It should be pointed out that Weinstein and co-workers' explanations for the cell cycle kinetics in psoriasis and their recommended therapeutic regimes using methotrexate are officially sponsored and distributed by the American Academy of Dermatology (Weinstein, 1971b, 1973). In addition, the systemic administration of methotrexate as a treatment for psoriasis has U.S. Federal Drug Administration approval (Roenigk, Maibach & Weinstein, 1973).

This is all based upon the assumption that the 457 h (normal) and the 37·5 h (involved psoriatic) epidermal cell cycle time values are correct. Recently a controversy has arisen between Weinstein (1975) and a group of British investigators, Goodwin, Hamilton & Fry (1975) who obtain a value of 91 h (or 111 h) for the cell cycle time of the germinative cells in involved psoriatic epidermis. In their controversy, both sets of authors allude to some of my publications on the subject. Because of this, and because of our extensive investigations of cell cycles in mammalian epidermis (see Gelfant, 1966; Gelfant & Candelas, 1972), this review was undertaken.

BACKGROUND INFORMATION ON OBTAINING CELL CYCLE VALUES BY THE LABELLED MITOSES METHOD

The duration of each period of the cell cycle can be measured by plotting the appearance of labelled mitoses in samples taken at hourly intervals after a single pulse exposure to ^{3}H-thymidine. Fig. 1 explains the principle and the procedure for timing the periods of the cell cycle using the labelled mitoses method. (The procedure can be used *in vitro*, in which case ^{3}H-thymidine is washed out of the system within an hour, or it can be used *in vivo*, in which case the injected ^{3}H-thymidine is

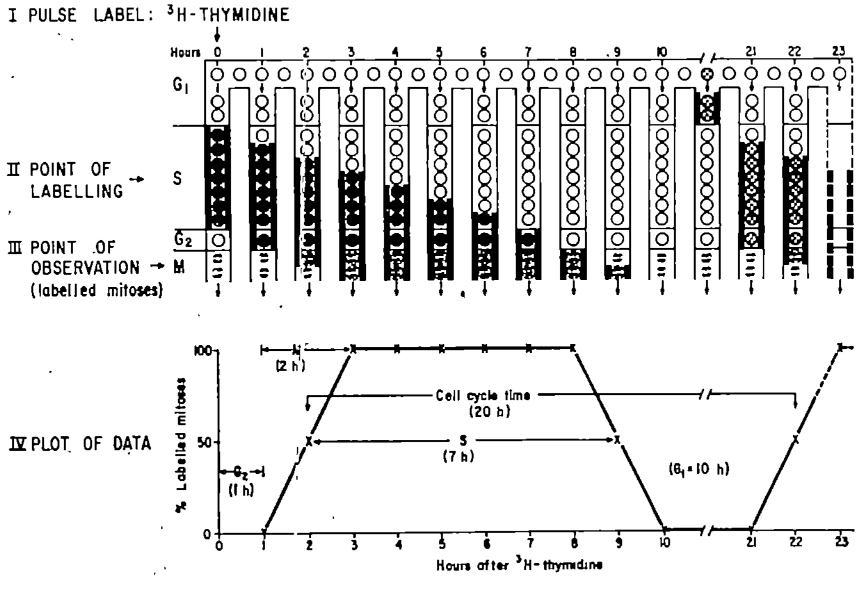


FIGURE 1. Explanation of the principle and the procedure for timing the periods of the cell cycle, using the labelled mitoses method. (The cell cycle time values are hypothetical and the data curves have been idealized.)

excreted within an hour—thus achieving pulse labelling in both cases.) The circles in the first column represent nuclei of cells asynchronously moving through the G_1, S, G_2 periods of the cell cycle. The experiment begins (I) by introducing a pulse label of ^{3}H-thymidine. Only cells in S(II) and all cells in the S period become labelled (as shown by the shaded circles). Thus, we now have a block of labelled cells (marked by shaded bars), all of which are in the S period. The principle of this procedure is to observe the movement in time of this labelled block of cells through a fixed point, M, where they come through as labelled mitoses (III), and then to determine the periods of the cell cycle from a plot of the data (IV)—usually referred to as a PLM (percent labelled mitoses) curve or a FLM (fraction labelled mitoses) curve.

The data are plotted as the percent labelled mitoses with time; that is, in samples taken at hourly intervals after the initial pulse label of ^{3}H-thymidine. One usually scores at least 100 mitoses in each sample at each interval in order to obtain meaningful data. The S period is measured from the 50% point on the ascending limb of the plotted curve, which represents the position of the front edge of the block of labelled cells moving through M at 2 h (see the 3rd column of cells at 2 h in the diagram above the plot of data) to the 50% point on the descending limb of the curve, representing the tail edge of the block of labelled cells moving through M at 9 h (as illustrated in the diagram). Thus, it took 7 h for the original labelled block of cells in S to move through the fixed point of observation, M. Since this is an asynchronous system, it will take any cell 7 h to move through S (that is to synthesize DNA).

The G_2 period is measured from the time the label was introduced at 0 h to the first appearance of a labelled mitosis (which was at 1 h). M is measured from the first appearance of a labelled mitosis at time 1 h, to the point where all mitoses come through labelled, at time 3 h (see diagram above plot which depicts the movement of the block of labelled cells through G_2 and M at hourly intervals). In this way, direct measurements are made of the S, G_2 and M periods.

The G_1 period cannot be measured directly from the plot of the data, but it is determined in the following manner: A direct measurement of the total cell cycle time is made from the 50% point of the ascending limb of the first wave of labelled mitoses (as illustrated in the diagram, the original block of labelled cells has gone through cell division; the daughter cells, containing ½ the amount of label, shown by lighter shaded circles, are moving through the cell cycle, and at 22 h, the leading edge of the labelled block of cells is again passing through the 50% point of M). Thus, the total cell cycle time is 20 h long. The G_1 period is calculated by subtracting S, G_2 and M (10 h) from the total cell cycle time (20 h); therefore, the G_1 period is 10 h.

The principle of obtaining a labelling index (LI) (no. labelled cells/no. cells counted, expressed in percent) is also illustrated in Fig. 1. A labelling index is based upon monitoring the number of cells moving through S at any given point in time. This is accomplished by introducing a pulse label of ^{3}H-thymidine, ending the experiment about 45 min later, and counting the number of labelled interphase nuclei in autoradiographs (see the diagram in the first column at 0 h depicting the result of a pulse label with ^{3}H-thymidine). For example, if 100 cells were counted and 7 were labelled, the LI would be 7%; if 50 cells were counted and 7 were labelled, the LI would be 14%, i.e. no. labelled cells/no. cells counted × 100.

The labelled mitoses method provides average or mean values (not absolute values) for timing the periods of the cell cycle. Its main disadvantage in human studies *in vivo* involves the direct measurement of total cell cycle time—which requires data on the second wave of labelled mitoses as depicted in Fig. 1. To obtain such data, one needs many short interval biopsy samples taken over long periods of time. It is because of this difficulty that total cell cycle time (Tgc) values on human epidermis *in vivo* are based upon mathematical equations and computer analyses of labelled mitoses curves (Steel & Haines, 1971; Gilbert, 1972) rather than on direct measurements of the total cell cycle time as described above. For additional information on the labelled mitoses procedure for timing the periods of the cell cycle, see Cameron (1971) and Steel (1973). For the most recent comprehensive review of cell cycle values in mammalian epidermis, see Potten (1975).

WEINSTEIN AND CO-WORKERS' EXPLANATION OF THE CELL CYCLE IN PSORIASIS

Equations for calculating germinative cell cycle time (Tgc) and labelling index (LI) (Weinstein & Frost, 1968)

$$\frac{\mathrm{Ns}}{\mathrm{Ts}} = \frac{\mathrm{Ngc}}{\mathrm{Tgc}}$$

Ns = number of cells in S; Ts = time (duration) of S; Ngc = total number of germinative cells (based on the assumption that 100% are cycling, i.e. are in the proliferative pool); Tgc = time (duration) of germinative cell cycle.

$$\text{Labelling Index (LI)} = \frac{\mathrm{Ns}}{\mathrm{Ngc}} \times 100$$

Ns = number of labelled germinative cells scored in autoradiographs and then *corrected up by a factor of 1·4.*

Actual calculations

Psoriatic epidermis (Weinstein & Frost, 1968). (Using LI of 22·7% for Ns and S period of 8·5 h for Ts.)

$$\frac{22{\cdot}7}{8{\cdot}5} = \frac{100}{x} \qquad \mathrm{Tgc} = 37{\cdot}5\ \mathrm{h}\ (1\tfrac{1}{2}\ \mathrm{days}).$$

Normal Epidermis (Weinstein, 1971b). (Using LI of 3·5% for Ns and S period of 16 h for Ts.)

$$\frac{3{\cdot}5}{16} = \frac{100}{x} \qquad \mathrm{Tgc} = 457\ \mathrm{h}\ (19\ \mathrm{days}).$$

Differences between psoriatic and normal epidermis (Weinstein *et al.*, 1971)

(1) Histologically thicker (5 times); (2) Larger germinative cell population (9 times); (3) More cells in S (6 times; LI 22·7 vs 3·5%); (4) Shorter duration of S period (2 times; 8·5 vs 16 h); (5) Faster germinative cell cycle time (12 times; 37·5 vs 457 h); (6) Faster epidermal cell transit time (7 times; 2 vs 14 days).

Clinical manifestation

Excessive scaling: up to 20 g of keratinous material can be shed in a 24 h period.

Reported cell cycle values

Table 1 traces the history and comments on the cell cycle values reported by Weinstein and co-workers since 1967. The key points are the labelling index and S period values—which are derived from experimental data, and which must be accurate for the Tgc (cell cycle time) values to be meaningful. It is clear from the comments and the footnote remarks that the LI value of 22·7% for psoriatic epidermis is questionable (primarily because of the 1·4 correction factor), and that both the LI value of 3·5% and the S period value of 16 h for normal epidermis are questionable (because no data were ever presented for the LI value, and the S period is drawn from a preliminary and inadequate labelled mitoses curve). Thus, on the basis of their own publications, Weinstein and co-workers' main conclusions regarding the germinative cell cycle times in psoriatic epidermis (37·5 h) and in normal epidermis (457 h) require further examination.

The following sections will examine the validity of using a 1·4 correction factor for determining labelling index data (this is a central issue in the Weinstein–Goodwin *et al.* controversy); we then will examine other reports of cell cycle values on psoriatic and normal epidermis.

THE 1·4 CORRECTION FACTOR

One of the main issues in the controversy between Weinstein and Goodwin *et al.* involves Weinstein's 1·4 correction method of determining the labelling index in human epidermis after a pulse injection of ^{3}H-thymidine. (This issue has already been brought up in the comments and footnote remarks in Table 1.) The controversy over the 1·4 correction factor can best be described by the following direct quotations from the published correspondence to the editors of the *British Journal of Dermatology* (1975) **92**, 229.

From Weinstein's letter:

> 'The principal difference in the two reports [Weinstein & Frost, 1968; Goodwin, Hamilton & Fry, 1974] lies in the labelling index (LI) determined for the germinative cell population of the psoriatic epidermis (labelled DNA cells/total germinative cells [counted]). We obtained a LI of 22·7%±2·4 (sd) while Goodwin *et al.* (1974) obtained half that value with a LI of 11·0%±1·7 (sd). The first reason for this difference is the use of the factor (1·4 × observed LI = true LI) in our study to compensate for the weak energy of tritium, limiting beta emissions to 1·5–2·0 microns. A correction factor of 1·4 is used based on the experimental studies of Iversen & Evensen (1962) and the earlier observations of Quastler & Sherman (1959). The factor can be derived from the calculations that in 6 μm thick histological sections, which cut through cells at variable levels, thirty of every 100 visible nuclei lie more than 2 μ beneath the surface of the tissue cut. Although these lower nuclei are visible microscopically, any tritium label contained in them does not have the energy to reach the photographic emulsion layered on the surface of the section and thus would not be recorded as labelled cells. The factor of 1·4 is used to compensate for this technical difficulty and raises the numerator of the LI fraction Ns/Ngc, where Ns is the number of labelled cells and Ngc is the number of germinative cells [counted]. Unfortunately, it is not technically feasible to cut human skin specimens at a thickness of 2 μ for autoradiographic studies.'

From Goodwin *et al.*, reply:

> 'The convention of multiplying the LI by a correction factor of 1·4 is no longer generally observed by cell biologists. Mention has already been made in the paper [Goodwin *et al.*, 1974] of the experiment carried out by Gelfant (1961) [misprint, should be 1962] who in his studies of mouse epidermis showed that the labelling indices obtained from 2 μ and from 7 μ thickness histological sections were the same. Therefore when using 5 μ sections this correction is unnecessary.'

Rather than argue the point or rely on our previous studies on mouse epidermis, the experiments in Table 2 were performed to answer the question of whether one needs to upgrade autoradiographic counts of ^{3}H-thymidine labelled nuclei in human epidermis because of using 6 μm histological sections. Skin samples embedded in paraffin blocks were kindly provided to us by Dr Jose Terz, Division of Surgical Oncology, Medical College of Virginia. The section thickness experiments in Table 2 were performed on three different irradiated skin biopsy samples taken at different times from a patient who was being infused with ^{3}H-thymidine for 13–15 days, and who was receiving radiation therapy for an extensive basal cell carcinoma of the face. In each experiment, two or three individual,

TABLE 1. Labelling index (LI), duration of S period (S) and duration of cell cycle (Tgc) values on psoriatic and normal epidermis reported by Weinstein & Co-workers, 1967–1975.

LI (%)	S (hours)	Tgc (hours)	Reference§	Comments*
				Psoriatic epidermis
22·7±2·4	8	37·5	1967 (1)	Statements in abstract; Tgc incorrectly calculated. According to values stated in this report, Tgc should be 35·2 h
22·7±2·4	8·5	37·5	1968 (2)	LI: Individual data, 7 patients; counts made from photographs; av. 850 germinative cells counted per patient; *data corrected up by a factor of 1·4*† S: Determined by labelled mitoses (LM) curve from biopsy samples from 8 patients; av. 113 mitoses counted for each patient at each interval on LM curve; S period measured between 37% points on LM curve
				Normal epidermis
5·6±1·7	8	144	1967 (1)	No details; no data; statements in abstract
5·2±1·5	8·5	163	1968 (2)	LI: No data presented for this value; statement in text; counts made from photographs; corrected up by a factor of 1·4(?)† so-called 'normal' epidermis included samples from patients with psoriasis and other cutaneous diseases. S: Used S value of psoriatic epidermis
5·2	16	308	1969 (3)	LI: Same value reported in ref. 2 S: Determined by LM curve from biopsy samples from 6 patients; authors do not provide information on the no. mitoses counted for each patient at each interval on LM curve. They imply that only small nos of mitotic figures were counted, and they consider LM curve to be preliminary data—requiring further confirmation
3·5	16	457	1971 (4)	LI: No data presented for this value; LI of 3·5% presented in text as having been reported in ref. 2. ref. 2 reports LI of 5·2%, not 3·5% S: No mention that this value is based upon preliminary data published in 1969 (3) Tgc: *Has now reached its maximum value of 457 h based upon incorrect LI value and unconfirmed S value*
3·5	16	457	1971–1973 (5–10)	Same LI, S, Tgc values as reported in Ref 4; no additional data presented in any of these papers; refs 2 & 4 used to support statements on LI, S, Tgc values. (Ref. 2 repeatedly incorrectly quoted; Ref. 4 presented no data on any of the values)
			Additional LI data normal epidermis	
3·0–3·8 (av 3·4)	—	—	1968 (11)	Individual data from 9 patients without cutaneous diseases, biopsy samples taken at 12 AM, 6 AM, 12 PM, 6 PM; counts made from photographs; *data not corrected up by a factor of 1·4*.† (No significant differences in LI at different hours)
3·1‡	—	—	1975 (12)	Individual data from 13 patients without cutaneous disease who had been injected with 0·05 ml saline 48 h prior to obtaining labelling indices; counts made by direct viewing under high-power magnification (not from photographs); approx. 2500 basal cells counted for each patient; *LI not expressed in percent*,‡ *data not corrected up by a factor of 1·4*†

---

* Data based upon single pulse ^{3}H-thymidine labelling *in vivo*. For explanations of labelling index, labelled mitoses curve and measurement of the S period, see Fig. 1. For calculation of Tgc values, see Weinstein & Frost's equations explained above.

† With regard to the 1·4 correction factor for histological section thickness, our results in Table 2 will demonstrate that there was no need to use this correction factor. However, if Weinstein and co-workers were interested in magnifying the differences in labelling indexes between psoriatic and normal epidermis, the 1·4 correction factor could be helpful. For example a corrected labelling index of 22·7% for psoriatic epidermis vs an uncorrected labelling index of 3·5% for normal epidermis magnifies the differences in the Tgc values as calculated by Weinstein's equation.

It is interesting to note that at the same time Weinstein argues for the validity of correcting his labelling index data by a factor of 1·4 (Weinstein, 1975) he publishes labelling index data which have not been corrected up by a factor of 1·4 (Eaglestein & Weinstein, 1975; Table ref. 12). It is also interesting to note that as far back as 1968,

TABLE 2. Experiments to test the validity of correcting the labelling index (LI) up by a factor of 1·4—to compensate for histological section thickness in human epidermis*

Sect. thickness (μm)	Av. no. labelled nuclei	Av. no. basal cells counted†	Av. LI (%)‡
	Experiment I		
3	576 ± 16	3482 ± 279	17·0 ± 1·3
6	672 ± 17	4008 ± 209	17·1 ± 1·1
	Experiment II		
3	215 ± 16	1999 ± 133	10·9 ± 0·8
5	266 ± 28	2188 ± 114	12·0 ± 0·8
7	284 ± 13	2662 ± 108	10·7 ± 0·4
	Experiment III		
3	125 ± 9	1389 ± 37	9·1 ± 0·8
5	161 ± 11	1579 ± 33	10·1 ± 0·5
7	179 ± 17	1856 ± 53	9·6 ± 0·8

* Experiments were performed on three different skin biopsy samples from a patient who had received ^{3}H-thymidine *in vivo*. Six separate slides of adjacent sections were cut, one at a time, for each experiment. The data represent average counts from six slides.

† Approximately 22,000 basal epidermal cells were counted for each section thickness in Experiment I; 14,000 for each section thickness in Experiment II and 10,000 for each section thickness in Experiment III.

‡ Average LI of six individual labelling indexes calculated at each point. LI = no. labelled nuclei/no. basal cells counted × 100. The LI in these experiments cannot be compared with other studies—which take biopsy samples 1 h after injection of ^{3}H-thymidine. In these experiments the subject had received ^{3}H-thymidine for longer periods of time.

---

and in the same volume of the *Journal of Investigative Dermatology*, Weinstein & Frost (Table ref. 2) report a labelling index for psoriatic epidermis which has been corrected up by a factor of 1·4; and Kahn, Weinstein & Frost (Table ref. 11) report labelling indexes for normal epidermis which have not been corrected up by a factor of 1·4.

‡ In this report by Eaglestein & Weinstein, labelling index is expressed as no. labelled nuclei per 50 basal epidermal cells—rather than using the conventional expression of labelling index in percent, i.e. no. labelled cells/no. germinative cells counted —100. Labelling index expressed in percent has been used by Weinstein and co-workers in all of their previous publications since 1968. If the above data (3·1 labelled cells/50 epidermal cells) were corrected up by a factor of 1·4, the labelling index would be 8·7%. And if the 8·7% labelling index were used to calculate the Tgc value according to Weinstein's equation $\left(\frac{8\cdot7}{16} = \frac{100}{x}\right)$ the Tgc for normal epidermis would be 184 h (not 457 h).

In their most recent publication (Berk, S.H., Pennys, N.S. & Weinstein, G.D. (1976) *Archives of Dermatology*, **112**, 485), Weinstein and co-workers report the following procedure for determining labelling indexes in human epidermis:

'The labelling index for each site was calculated by dividing the mean number of labelled cells (times 100) by the mean number of basal cells. No corrections were made for histologic tissue thickness (cut at 6μ) or for the tritium energy.'

§ *References*

1. WEINSTEIN, G.D. & FROST, P. (1967) *Clinical Research*, **15**, 256.
2. WEINSTEIN, G.D. & FROST, P. (1968) *Journal of Investigative Dermatology*, **50**, 254.
3. WEINSTEIN, G.D. & FROST, P. (1969) *National Cancer Institute Monograph*, **30**, 225.
4. WEINSTEIN, G.D., VELASCO, J. & FROST, P. (1971) In: *Psoriasis* (Ed. by E.M.Farber and A.J.Cox), p. 393. Stanford University Press, Stanford, California.
5. WEINSTEIN, G.D. & FROST, P. (1971) *Archives of Dermatology*, **103**, 33.
6. WEINSTEIN, G.D., GOLDFADEN, G. & FROST, P. (1971) *Archives of Dermatology*, **104**, 236.
7. WEINSTEIN, G.D. (1971a) *Annals of the New York Academy of Science*, **186**, 452.
8. WEINSTEIN, G.D. (1971b) National Program for Dermatology, American Academy of Dermatology, Part I (an autotutorial program).
9. WEINSTEIN, G.D. & MCCULLOUGH, J.L. (1973) *Annual Review of Medicine*, **24**, 345.
10. WEINSTEIN, G.D. (1973) National Program for Dermatology, American Academy of Dermatology, Part II (an autotutorial program).
11. KAHN, G., WEINSTEIN, G.D. & FROST, P. (1968) *Journal of Investigative Dermatology*, **50**, 459.
12. EAGLESTEIN, W.H. & WEINSTEIN, G.D. (1975) *Journal of Investigative Dermatology*, **64**, 386.

TABLE 3. Other reports of labelling index (LI), duration of S period (S), and duration of cell cycle (TgC) values on psoriatic and normal epidermis

LI (%)	S (hours)	Tgc (hours)	Reference‡	Comments†
				Psoriatic epidermis
	In vivo			
11	9·8	91	(7)	Single pulse ^{3}H; individual data, 8 patients; counts made by direct viewing under high-power magnification LI: based on av. counts of approx. 3000 germinative cells per patient; *data not corrected up by a factor of 1·4* S: 9·8 h from computer analysis of LM curve, 12·2 h from direct measurement between 37% points on LM curve; av. 221 mitoses counted for each patient at each interval on LM curve. Tgc value of 111 h calculated from Weinstein's equation $\left(\frac{11}{12\cdot2}=\frac{100}{x}\right)$
(11)	(12·2)	(111)		
10	7·7	56	(5)	LI: Single pulse ^{3}H; individual data, 6 patients; counts made by direct viewing under oil immersion; 8000 germinative cells counted for each biopsy; *data not corrected up by a factor of 1·4* S & Tgc: From Filbert program computer analysis of LM curve in 1 patient; biopsy samples taken at intervals up to 45 h after pulse labelling; 100 mitoses counted for each biopsy. Tgc value of 77 h calculated from Weinstein's equation $\left(\frac{7\cdot7}{0}=\frac{100}{x}\right)$
(10)	(7·7)	(77)		
	In vitro			
2·6 (uninvolved) 4·1 (involved)	— —	— —	(11)	*In vitro* incubation ^{3}H, 24 h; autoradiographs exposed for 3 days; individual data, 15 patients
4·6 (uninvolved)	—	—	(13)	*In vitro* incubation ^{3}H, 60 min; no individual patient data
11·7	—	—	(17)	*In vitro* incubation ^{3}H, 60 min (LI decreases after 120–180 min of incubation)
27·5 (scalp)	—	—	(18)	*In vitro* incubation ^{3}H, 2½ h; 13 patients, no individual patient data
13·6	14·4	114	(16)	*In vitro* incubation double labelling, ^{3}H: ^{14}C; individual data, 5 patients
12	6·5	53·5	(3)	*In vitro* tissue culture ^{3}H (7-day out-growth cultures). Tgc values obtained from direct measurements of two successive labelled mitoses curves
	Other procedures			
13·6 (involved) 11·6 (uninvolved)	— —	— —	(14)	Biopsy samples of involved and uninvolved psoriatic skin cultured in diffusion chambers in peritoneal cavity of adult mice; ^{3}H, 1 h
—	15	50	(2)	Epidermal cell suspensions from skin biopsies analysed by impulse cytophotometry. S value does not represent h, but is derived from % of cells in the different phases of cell cycle (*i.e.* G_1, 79%; S, 15%; G_2 & M, 6%)
				Normal epidermis
	In vivo			
3·8	7·6	206	(1)	Double labelling, ^{3}H: ^{3}H; individual data, 6 patients; counts made by direct microscopic viewing under high-power magnification; LI and S values *corrected up by a factor of 1·4*
4·9 ± 1·1	—	—	(6)	Single pulse ^{3}H; no data, statement in text; biopsy samples from 21 volunteers; counts made by direct microscopic viewing under high-power magnification; LI based on counts of approx. 6000 germinative cells per patient; *LI not corrected up by a factor of 1·4*, authors say they could not determine the duration of S by a labelled mitoses curve because of the lack of sufficient mitotic figures in biopsy samples of normal epidermis

TABLE 3 (*cont.*)

	In vitro			
0·9	—	—	(11)	*In vitro* incubation ^3H, 24 h; autoradiographs exposed for 3 days; individual data, 13 patients
2·3	—	—	(13)	*In vitro* incubation ^3H, 60 min; no individual patient data
8·0 (body)	—	—	(18)	*In vitro* incubation ^3H, 2¼ h; 9 patients, no individual patient data
9·5 (scalp)	—	—	(18)	
2·4	6·6	282	(16)	*In vitro* incubation double labelling, ^3H: ^{14}C; individual data, 3 patients
4·5	7·6	170	(10)	*In vitro* double labelling, ^3H:^3H; individual data, 3 patients
4·8	10	213	(8)	*In vitro* double labelling, ^3H:^3H; individual data, 6 patients
5·8	10·3	184	(9)	*In vitro* incubation double labelling, ^3H:^3H; individual data, 3 patients
8·6	5·9	142–166	(4)	*In vitro* incubation double labelling, ^3H:^3H
10·5	11·5	59	(3)	*In vitro* tissue culture ^3H (7-day out-growth cultures). Tgc values obtained from direct measurements of two successive labelled mitoses curves
	Other procedures			
—	6	50	(2)	Epidermal cell suspension from skin biopsies analysed by impulse cytophotometry. S value does not represent h, but is derived from % of cells in the different phases of cell cycle (i.e. G_1, 88%; S, 6%; G_2 & M, 6%)

In vivo–in vitro comparisons (LI)

Labelling index (%)				
In vivo	*In vitro*	Autoradiographic exposure time (days)		
1·62	0·97	3	(12)	Normal epidermis, individual data, 6 patients; ^3H *in vivo*, 40 min; *in vitro* incubation, 4 h
2·5	1·37	7		
2·2	2·3	14–56	(15)	Normal epidermis, individual data, 6 patients; ^3H *in vivo*, 1 h; *in vitro* incubation, 2 h

---

* Tgc as reported by the authors; not calculated according to Weinstein's equation.

† Data based upon a variety of *in vivo* and *in vitro* labelling procedures using ^3H and ^{14}C-thymidine. For explanation of data obtained by single pulse ^3H-thymidine drawn from labelled mitoses curves, see Fig 1. For explanation of double labelling methods, see Wimber & Quastler (1963) and Pilgrim & Mauer (1965).

‡ *References*

1. ALLEGRA, F. & DEPANFILIS (1974) *Acta dermato-venereologica*, **54**, 87.
2. BAUER, F.W. & DE GROOD, R.M. (1975) *British Journal of Dermatology*, **93**, 225.
3. CHOPRA, D.P. & FLAXMAN, B.A. (1974) *Cell and Tissue Kinetics*, **7**, 69.
4. CHRISTOPHERS, E. & SCHAUMLOFFEL, E. (1967) *Archiv für klinische und experimentelle Dermatologie*, **228**, 57.
5. DUFFILL, M., WRIGHT, N. & SHUSTER, S. (1976) *British Journal of Dermatology*, **90**, 355.
6. EPSTEIN, W.L. & MAIBACH, H.I. (1965) *Archives of Dermatology*, **92**, 462.
7. GOODWIN, P., HAMILTON, S. & FRY, L. (1974) *British Journal of Dermatology*, **90**, 517.
8. HEENAN, M. & GALAND, P. (1971) *Journal of Investigative Dermatology*, **56**, 425.
9. HEENAN, M., ACHTEN, G. & GALAND, P. (1973) *Cancer Research*, **33**, 123.
10. HEENAN, M., LAMBERT, J.C., ACHTEN, G. & GALAND, P. (1975) *Journal of the National Cancer Institute*, **54**, 825.
11. HELL, E. & HODGSON, C. (1966) *British Journal of Dermatology*, **78**, 262.
12. HELL, E. & MAIBACH, H. (1972) *British Journal of Dermatology*, **86**, 506.
13. KAIDBEY, K.H. & KURBAN, A.K. (1971) *British Journal of Dermatology*, **85**, 162.
14. KARINIEME, A-L. (1974) *Journal of Investigative Dermatology*, **63**, 388.
15. LACHAPELLE, J.M. & GILLMAN, T. (1969) *British Journal of Dermatology*, **81**, 603.
16. PULLMANN, H., LENNARTZ, K.J. & STEIGLEDER, G.K. (1974) *Archiv für dermatologische Forschung*, **250**, 177.
17. SCHMID, G.H. & HORNSTEIN, O.P. (1974) *Virchows Archiv, Abteilung B, Zellpathologie*, **15**, 165.
18. SHAHRAD, P. & MARKS, R. (1976) *British Journal of Dermatology*, **90**, 7.

adjacent sections were cut, one at a time, at the different section thicknesses or nominal microtome settings (listed in Table 2) and placed on the same slide. This was repeated six times (6 slides for each experiment) so that the counts could be subjected to statistical analysis. The slides were dipped in Kodak NTB 3 liquid emulsion, exposed at 4°C for 7½ weeks, processed and stained with haematoxylin and eosin.

The results of the experiments in Table 2 are clear. The procedure of using a 1·4 correction factor to compensate for inadequate autoradiographic labelling in 6 μm sections of human epidermis is not valid; this procedure is unnecessary and it is incorrect. Experiments such as these should have been performed by Weinstein & Frost (1968) before they insisted on multiplying their labelled nuclei counts by 1·4 (also it is obviously 'feasible to cut human skin specimens at a thickness of 3 μm for autoradiographic studies'). In summary, the results in Table 2 show no statistically significant differences in labelling indexes in any of the three separate experiments in which adjacent histological sections were cut at either 3, 5, 6 or 7 μm.

OTHER REPORTS ON CELL CYCLE VALUES IN PSORIATIC AND IN NORMAL EPIDERMIS

Table 3 looks into other published reports of cell cycle time values for psoriatic and normal human epidermis. The purpose of the Table is to compare the results of other investigators with those of Weinstein & co-workers—primarily to focus on their 37·5 h (psoriatic) and 457 h (normal) epidermal cell cycle times. The data in Table 3 are based upon a variety of DNA labelling procedures with ^{3}H or ^{14}C-thymidine in single or double labelling regimes, *in vivo* or *in vitro*. These various methods are employed because of the inherent difficulty in measuring total cell cycle time in human subjects *in vivo*. (See section on background information, Fig. 1).

The cell cycle values for psoriatic epidermis reported by Goodwin *et al.* in Table 3 (ref. 7) are those disputed by Weinstein (in his letter to the *British Journal of Dermatology*, 1975). Both sets of authors use the same general procedure for obtaining their data, i.e. ^{3}H-thymidine pulse labelling and biopsy sampling *in vivo*. Their reported differences in labelling indices (22·7 vs 11%) can be explained by Weinstein & Frost's procedures of (1) upgrading their labelled cell counts by a factor of 1·4, (2) by making their counts from photomicrographs of histological sections—rather than by direct viewing under a microscope, and (3) by counting relatively small numbers of germinative cells in their biopsy samples (only 850 vs 3000 counted by Goodwin *et al.*). The most recent *in vivo* study by Duffill, Wright & Shuster (1976) (ref. 5, Table 3) confirms the labelling index obtained by Goodwin *et al.* If the data of Goodwin *et al.* and Duffill *et al.* are calculated according to Weinstein's equation, the total cell cycle time (Tgc) for psoriatic epidermal germinative cells is either 111 or 77 h (not 37·5 h)—as measured by pulse labelling with ^{3}H-thymidine *in vivo*.

It is interesting to note that in two reports of relatively short Tgc values (53·5 h in an *in vitro* tissue culture system (ref. 3) and 50 h when psoriatic biopsy samples are analysed by impulse cytophotometry (ref. 2)) that there are no differences in Tgc values between psoriatic and normal epidermis.

With regard to the Tgc values for normal epidermis in Table 3, none of them support Weinstein's figure of 457 h. Based upon the values obtained by a variety of cell cycle timing procedures, a reasonable estimate for the germinative epidermal cell cycle time in normal epidermis *in vivo* would be around 200 h—or about twice the figure settled on for psoriatic epidermis (above). An additional point of interest in Table 3 involves the possibility that the S period in psoriatic epidermal cells is *longer not shorter* than in normal epidermis. This is shown in a number of reports which record the S period of both psoriatic and normal epidermal cells by the same procedures (see refs. 2 & 16, Table 3). Further evidence for this possibility is shown in a recent report by Heenan *et al.* (1975) who find an increased S phase duration in several premalignant tumours in human epidermis.

THE CELL CYCLE IN PSORIASIS: A REAPPRAISAL AND A NEW CONCEPT

It is clear from the above analysis of the cell cycle values reported by Weinstein and co-workers and by other investigators that the germinative cell cycle time in psoriatic epidermis is not speeded up or shortened by a factor of 12 (presumably reduced from 457 h in normal epidermis to 37·5 h in psoriatic epidermis). On the basis of the studies of other investigators reviewed above, the germinative cell cycle time in normal epidermis *in vivo* is probably closer to 200 h (not 457 h), and in psoriatic epidermis it is probably around 100 h (not 37·5 h); *a two-fold difference*. (Indeed, several studies, Table 3, indicate no differences in Tgc values between normal and psoriatic epidermis.) Thus, Weinstein and co-workers' explanation that excessive psoriatic scaling is a result of a 12-fold acceleration in germinative epidermal cell cycle time is unacceptable.

There is, nevertheless, increased epidermal cell proliferation in active lesions of psoriasis—as observed by increases in labelling index and mitotic index counts (Goodwin, Hamilton & Fry, 1973).

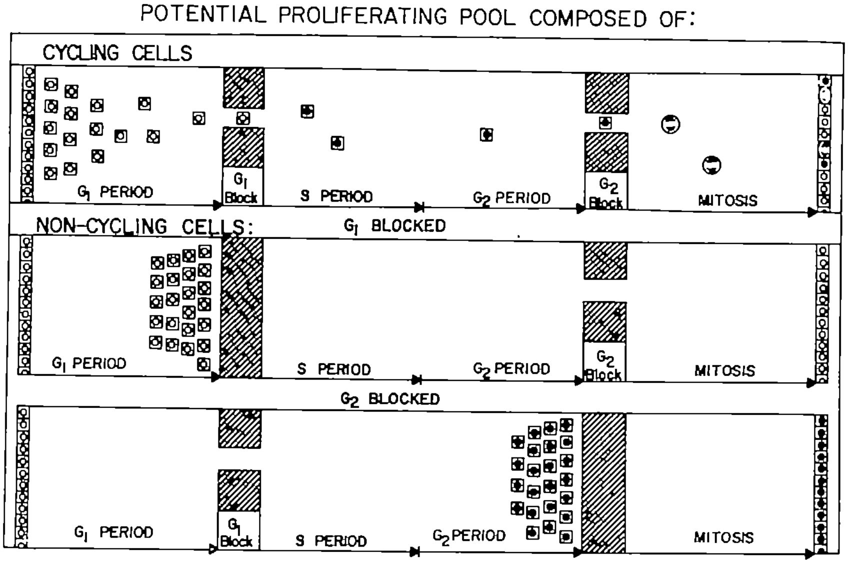


FIGURE 2. Concept of cycling and non-cycling cells existing within the stratum germinativum of human epidermis (after Gelfant & co-workers, 1963–72; see footnote for refs.). The ideas depicted here are also applicable to all plant, animal, and tumour tissues capable of proliferation.

The idea depicted in Fig. 2 of G_1 and G_2 blocks was introduced by Gelfant (1963) based upon studies in mouse epidermis. He visualized and provided evidence for two inherent blocks in the cell cycle: A G_1 block between the G_1 and the S periods; and a G_2 block between G_2 and the period of mitosis. That the blocks are located at the $G_1 \rightarrow S$ and $G_2 \rightarrow M$ transition points, rather than between S and G_2 or between M and G_1 is also supported by the generalized effects of irradiation or carbohydrate starvation in animal or plant tissues. In both instances, cells are blocked in G_1 or in G_2, but not in S or in M (Lesher & Lesher, 1972; Van't Hof, 1968). The terms 'cycling' and 'non-cycling' cells were introduced by DeCosse & Gelfant (1968). Using a combined autoradiographic–Feulgen microspectrophotometric procedure, they identified and separated cycling from two classes of non-cycling cells in actively proliferating Ehrlich ascites tumour cells. Conclusive evidence for the idea that non-cycling cells can remain in the G_2 period indefinitely was presented by Pederson & Gelfant (1970). They labelled mice continuously with ^{3}H-thymidine for more than 6 months and then stimulated regeneration in ear epidermis and in kidney tubular epithelium. The appearance of unlabelled mitoses in both the epidermis and the kidney tubules was evidence that non-cycling G_2 blocked cells can remain held up in the G_2 period indefinitely. The appearance of labelled mitoses, particularly in the kidney, was evidence for non-cycling cells—having the capacity to hold up in the G_1 period indefinitely. For additional information and other examples which support the concepts in Fig. 2, see Gelfant (1966), Gelfant & Candelas (1972), and Gelfant & Smith (1972).

How does one explain this increased proliferative activity if it is not due to a dramatic shortening in the cell cycle time of the germinative epidermal cells? The purpose of this section is to answer this question by the concept depicted in Fig. 2. This concept is based upon the idea of two inherent blocks in the cell cycle; a G_1 block between the G_1 and S periods, and a G_2 block between G_2 and the period of mitosis. What is being proposed in Fig. 2 is that the stratum germinativum in normal and in psoriatic epidermis (depicted as a single layer of cells in the diagram) is composed of not one, but three categories of potentially proliferating cells—each having different patterns of cell division as seen in relation to the G_1 and G_2 blocks.

The first is cycling cells that are actively moving through the cell cycle. The G_1 and the G_2 blocks in these cells are partially open, implying various physiological states of expansion or retraction. Cycling cells can be identified in the stratum germinativum as cells in interphase having nuclear DNA contents of 2C (if they are in the G_1 period, unshaded circles) or 4C (if they are in the G_2 period, shaded circles); intermediate DNA contents between 2C and 4C would represent cells in the S period (2C and 4C are cytochemical terms which refer to diploid and tetraploid 'classes' of DNA contents—corresponding to 2N and 4N diploid and tetraploid 'number' of chromosome sets, terms used by cytogeneticists). Cycling cells can be identified in the S period where they incorporate ^{3}H-thymidine and appear as cells with labelled nuclei in autoradiographs, and they also can be seen in the various stages of visible mitosis (depicted as rounded cells in anaphase, Fig. 2). In addition, there are two categories of non-cycling cells, G_1 blocked and G_2 blocked, which are capable of moving into the cell cycle upon specific stimulation. G_1 blocked cells exist only in interphase as 2C-DNA content cells (unshaded circles); G_2 blocked cells also exist only in interphase, but they have 4C-DNA content nuclei (shaded circles). Non-cycling cells can remain G_1 or G_2 blocked indefinitely. All three populations of cycling, non-cycling-G_1 blocked, and non-cycling-G_2 blocked cells exist within the stratum germinativum of normal, involved and uninvolved psoriatic epidermis; and they represent the potential proliferating pools in these tissues. Their relative proportions and transitions vary with the changing physiological and clinical manifestations involved in psoriasis. Thus, increased epidermal cell proliferation in active lesions of psoriasis would be brought about, to a large extent, by a proliferative recruitment or a release of non-cycling G_1 and G_2 blocked cells—resulting in the force responsible for the excessive scaling in psoriasis. (In a recent report, Marks [1975] reviews the relationship between epidermal cell proliferation and scaling in a number of skin diseases—other than psoriasis. Although there is a positive correlation in most red and scaly skin diseases, there are some scaling conditions which are not associated with a high rate of epidermal cell proliferation.)

There is some direct evidence for the existence of non-cycling cells in human epidermis. Although the results are considered to be preliminary and were obtained from irradiated skin, the biopsy sample in Experiment 1, Table 2 came from a patient who had been infused continuously with ^{3}H-thymidine for 15 days, and yet only 17% of the basal cells were labelled. If all of the germinative cells were cycling, then all basal cells would be labelled after 15 days of continuous exposure to ^{3}H-thymidine. (With a total cell cycle time of 8 days [200 h], all cycling cells would have had the opportunity to incorporate ^{3}H-thymidine at least once during the 15 day exposure period.) Thus, the results of this experiment indicate that there is a relatively large fraction of non-cycling cells in human epidermis. (Speculatively, more than 60% of the germinative cells in normal epidermis may be non-cycling; 90% of these in the G_1 blocked state and 10% in the G_2 blocked state.)

A NEW APPROACH FOR THE STUDY AND TREATMENT OF PSORIASIS

The idea that the germinative cells in normal and in uninvolved psoriatic epidermis (during clinical remission) exist primarily in the non-cycling G_1 and G_2 blocked states, leads to the suggestion that one focus attention on non-cycling cells for the control and treatment of psoriasis. It may eventually turn out that the effective therapy for psoriasis is not to treat the patient during psoriatic flare-ups (with drugs which kill cycling cells), but rather that effective therapy be administered during periods of clinical remission by applying factors which would keep the germinative cells in the non-cycling state. We are currently developing a list of natural and experimental factors which open and close the G_1 and G_2 blocks of the cell cycle. Factors such as wounding and trauma, temperature, diet, hormones, tissue substances, other regulatory molecules, drugs and research biochemicals are being considered in this respect and also as potential agents for cancer therapy.

ACKNOWLEDGMENT

I would like to thank Mrs Elizabeth Purvis, secretary, and Mr James Parkerson, technician, for service beyond the call of duty. This work was supported by NIH Research Grants AM 16060 and AG 00460.

REFERENCES

CAMERON, I.L. (1971) Cell proliferation and renewal in the mammalian body. In: *Cellular and Molecular Renewal in the Mammalian Body* (Ed. by I.L.Cameron and J.D.Thrasher), p. 45. Academic Press, New York.

DECOSSE, J.J. & GELFANT, S. (1968) Noncycling tumor cells: Mitogenic response to antilymphocytic serum. *Science*, **162**, 698.

DUFFILL, M., WRIGHT, N. & SHUSTER, S. (1976) The cell proliferation kinetics of psoriasis examined by three *in vivo* techniques. *British Journal of Dermatology*, **94**, 355.

GELFANT, S. (1963) A new theory on the mechanism of cell division. *International Society for Cell Biology*, **2**, 229.

GELFANT, S. (1966) Patterns of cell division: the demonstration of discrete cell populations. In: *Methods in Cell Physiology, Vol. II*, p. 359. Academic Press, New York.

GELFANT, S. & CANDELAS, G.C. (1972) Regulation of epidermal mitosis. *Journal of Investigative Dermatology*, **59**, 7.

GELFANT, S. & SMITH, J.G., JR (1972) Aging: Noncycling cells an explanation. *Science*, **178**, 357.

GILBERT, C.W. (1972) The labelled mitoses curve and the estimation of the parameters of the cell cycle. *Cell and Tissue Kinetics*, **5**, 53.

GOODWIN, P., HAMILTON, S., & FRY, L. (1973) A Comparison between DNA synthesis and mitosis in uninvolved and involved psoriatic epidermis and normal epidermis. *British Journal of Dermatology*, **89**, 613.

GOODWIN, P., HAMILTON, S. & FRY, L. (1975) (Correspondence to the Editor, A Reply) *British Journal of Dermatology*, **92**, 230.

HEENAN, M., LAMBERT, J.C., ACHTEN, G. & GALAND, P. (1975) Kinetics of cell proliferation in benign and premalignant tumors of the human epidermis. *Journal of the National Cancer Institute*, **54**, 825.

LESHER, J. & LESHER, S. (1972) Effects of single dose, whole-body ^{60}Co gamma irradiation on number of cells in DNA synthesis and mitosis in the mouse duodenal epithelium. In: *Experimental Control of Mitosis: I*, p. 9. MSS Information Corporation, New York.

MARKS, R. (1975) Is there a relationship between clinical morphology and epidermal cell kinetics? *Proceedings of the Royal Society of Medicine*, **68**, 161.

PEDERSON, T. & GELFANT, S. (1970) G2-population cells in mouse kidney and duodenum and their behavior during the cell division cycle. *Experimental Cell Research*, **59**, 32.

PILGRIM, C.H. & MAUER, W. (1965) Autoradiographische Untersuchung über die Konstanz der DNS-verdopplungs-dauer bei Zellarten von Maus und Ratte Durch doppelmarkierung mit H^3- und ^{14}C-thymidin. *Experimental Cell Research*, **37**, 183.

POTTEN, C.S. (1975) Epidermal cell production rates. *Journal of Investigative Dermatology*, **65**, 488.

ROENIGK, H.H., JR, MAIBACH, H.I. & WEINSTEIN, G.D. (1973) Methotrexate therapy for psoriasis guideline revisions. *Archives of Dermatology*, **108**, 35.

STEEL, G.G. (1973) The measurement of the intermitotic period. In: *The Cell Cycle in Development and Differentiation* (Ed. by M.Balls and F.S.Billett), p. 13. Cambridge University Press, Cambridge.

STEEL, G.G. & HANES, S. (1971) The technique of labelled mitoses: Analysis by automatic curve-fitting. *Cell and Tissue Kinetics*, **4**, 93.

VAN'T HOF, J. (1968) Control of cell progression through the mitotic cycle by carbohydrate provision. *The Journal of Cell Biology*, **37**, 773.

WEINSTEIN, G.D. (1971a) Biochemical and pathophysiological rationale for amethopterin in psoriasis. *Annals of the New York Academy of Sciences*, **186**, 452.

WEINSTEIN, G.D. (1971b) Cell reproduction in skin diseases. Part I. Cell proliferation kinetics. National Program for Dermatology. American Academy of Dermatology. (An autotutorial program.)

WEINSTEIN, G.D. (1973) Cell reproduction in skin diseases. Part II. Chemotherapy of psoriasis based on cellular kinetics, National Program for Dermatology. American Academy of Dermatology. (An autotutorial program.)

WEINSTEIN, G.D. (1975) On the cell cycle of psoriasis (Correspondence to the Editor). *British Journal of Dermatology*, **92**, 229.

WEINSTEIN, G.D. & FROST, P. (1968) Abnormal cell proliferation in psoriasis. *Journal of Investigative Dermatology*, **50**, 254.

WEINSTEIN, G.D. & FROST, P. (1971) Methotrexate for psoriasis. *Archives of Dermatology*, **103**, 33.

WEINSTEIN, G.D. & MCCULLAGH, J.L. (1976) Cytokinetics and chemotherapy of psoriasis. *Journal of Investigative Dermatology*, **67**, 26.

WEINSTEIN, G.D., VELASCO, J. & FROST, P. (1971) Biological and pharmacological relationships of psoriasis and methotrexate. In: *Psoriasis* (Ed. by E.M.Farber and A.J.Cox), p. 393. Stanford University Press, Stanford, California.

WIMBER, D.E. & QUASTLER, H. (1963) A ^{14}C- and ^{3}H-thymidine double labeling technique in the study of cell proliferation in Tradescantia root tips. *Experimental Cell Research* **30**, 8.

In contrast to the cAMP catalyzing adenyl cyclase our knowledge about the cAMP degradative PDE is poor. Härkönen, Hopsu-Havu & Raij (1974) found the activity of PDE considerably lower in psoriatic plaques than in healthy epidermis. Voorhees *et al.* (1973) reported no differences in the activity of PDE between normal and psoriatic epidermis. Since the phosphodiesterase represents a possible target for influencing the intracellular cAMP level by chemical means, it seems necessary to clarify this point. For instance, in psoriasis it may be of therapeutic value to inhibit the cAMP degradation by PDE using caffeine or theophylline (Orfanos & Mahrle, 1976).

The purpose of this study was to obtain more information on the ultrastructural localization of the phosphodiesterase and to compare its activity in normal and psoriatic human skin.

MATERIALS AND METHODS

Four or six mm punch biopsies were taken from untreated psoriatic lesions of two patients and from normal skin of two healthy volunteers under local anaesthesia.

For the demonstration of phosphodiesterase activity we used a cytochemical method first applied by Shanta *et al.* (1966) as modified by King *et al.* (1974). The principle of this method was a two step reaction sequence (Fig. 1). The incubation medium contained cAMP as substrate for PDE forming 5'AMP, and 5' nucleotidase, i.e. snake venom, for the release of inorganic phosphate from 5'AMP. The inorganic phosphate was then precipitated by lead nitrate as an electron dense reaction product.

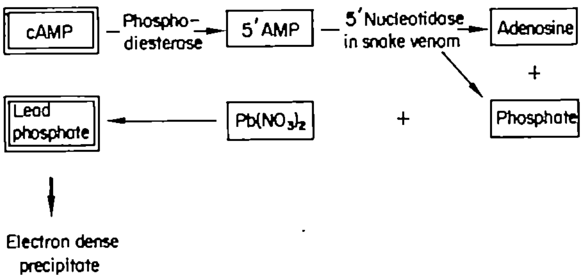


FIGURE 1. Reaction sequence in the phosphodiesterase method.

In detail, the procedure used was the following: the tissue blocks were prefixed at 4°C in a fixative solution consisting of 4% paraformaldehyde, 2·5% glutaraldehyde and 0·05% $CaCl_2$ buffered with 0·1 M cacodylate buffer, at pH 7·2 for 4 h. During prefixation the specimens were sliced with razor blades and they were then rinsed in cold cacodylate buffer. The incubation was performed in a medium containing 3 mM cAMP, 2mM $MgCl_2$, 2mM $Pb(NO_3)_2$, 5 mg/10 ml snake venom in 80 mM Tris-maleate buffer, at pH 7·4. The incubation time was 30 min in a water bath at 30°C.

After incubation all tissue flakes were thoroughly rinsed in acetate–Veronal buffer, pH 7·2, fixed in 1% osmic acid solution buffered with acetate–Veronal buffer, dehydrated in rising concentrations of acetone, and were then embedded in Araldite. Ultrathin sections were examined in a Zeiss EM 9A electron microscope before and after staining with uranyl acetate and lead citrate.

Controls were incubated in a medium (1) without the substrate cAMP, (2) without snake venom, (3) in addition with 50 or 200 mM theophylline as a specific PDE inhibitor and (4) in Tris-maleate buffer, which contained 2 mM $Pb(NO_3)_2$ only.

British Journal of Dermatology (1976) **95**, 591.

Localization and activity of tissue bound cyclic nucleotide phosphodiesterase in normal and lack of changes in psoriatic human skin*

G.MAHRLE AND C.E.ORFANOS

Departments of Dermatology, University of Göttingen, and University of Cologne, West Germany

Accepted for publication 4 June 1976

SUMMARY

This study has been undertaken to elucidate the localization and the activity of cyclic nucleotide phosphodiesterase (PDE) in psoriatic epidermis compared to normal. The results showed that the evaluation of cytochemical methods may be difficult because of the various factors which interfere with the reaction and the considerable amount of background staining. Additionally, only the tissue bound particulate enzyme fraction may be demonstrated by cytochemical means. Nevertheless, the method did reveal that the activity of PDE, if any, is localized on the cytoplasmic membranes of the cells, independent of their origin, and not on the cell surface. Moreover, no differences were found between normal and psoriatic skin. It seems, therefore, that the intracellular degradation of cAMP remains unaltered in psoriasis.

It is now well documented that the cyclic nucleotides cAMP and cGMP† as 'second messengers' control cell metabolism and cell growth. In psoriasis it is assumed that there is an imbalance in the cellular concentration of these two nucleotides with decreased cAMP and increased cGMP level (Voorhees, Stawiski & Duell, 1973). cAMP is synthesized from ATP by the enzyme *adenyl cyclase* and is subsequently broken down to adenosine 5′-monophosphate by the enzyme *cyclic nucleotide phosphodiesterase (PDE)*. In other words, the intracellular concentration of cAMP depends on the relative activities of these two enzymes.

Under the electron microscope the response of the membrane bound adenyl cyclase to catecholamines and glucagon is much lower in psoriatic epidermis than in normal (Mahrle & Orfanos, 1975). Thus, it seems that in psoriatic epidermis the intracellular cAMP concentration is uncoupled from hormonal control via cell membrane (Orfanos & Mahrle, 1976).

---

* Presented at the 3rd European Meeting on Electron Microscopy Applied to Cutaneous Pathology, 3–4 April 1976, Brussels, Belgium.

† Abbreviations used: PDE = 3′,5′-cyclic nucleotide phosphodiesterase; cAMP = cyclic adenosine 3′,5′-monophosphate; cGMP = cyclic guanosine 3′,5′-monophosphate; ATP = adenosine triphosphate; 5′-AMP = adenosine 5′-monophosphate.

RESULTS

After incubation in the complete standard medium the reaction product was exclusively localized in the cytoplasm of dermal and epidermal cells. Electron dense granular precipitates were regularly bound to cytoplasmic membranes of the ergastoplasm and the Golgi zone (Fig. 2). The mitochondria, the melanosomes and sometimes the nuclear membranes were also stained. On the other hand, the cell surface and the intercellular spaces were free from reaction product, except for some irregular and sparse contamination. In all specimens no differences were seen between normal and psoriatic skin.

As a rule, all cell types showed the same cytochemical pattern, for instance keratinocytes, Langerhans cells, endothelial cells, and fibrocytes. Particularly in the Langerhans cells prominent lead salt deposits were also found in the racket-shaped Langerhans granules (Figs 3 and 4). The intensity of

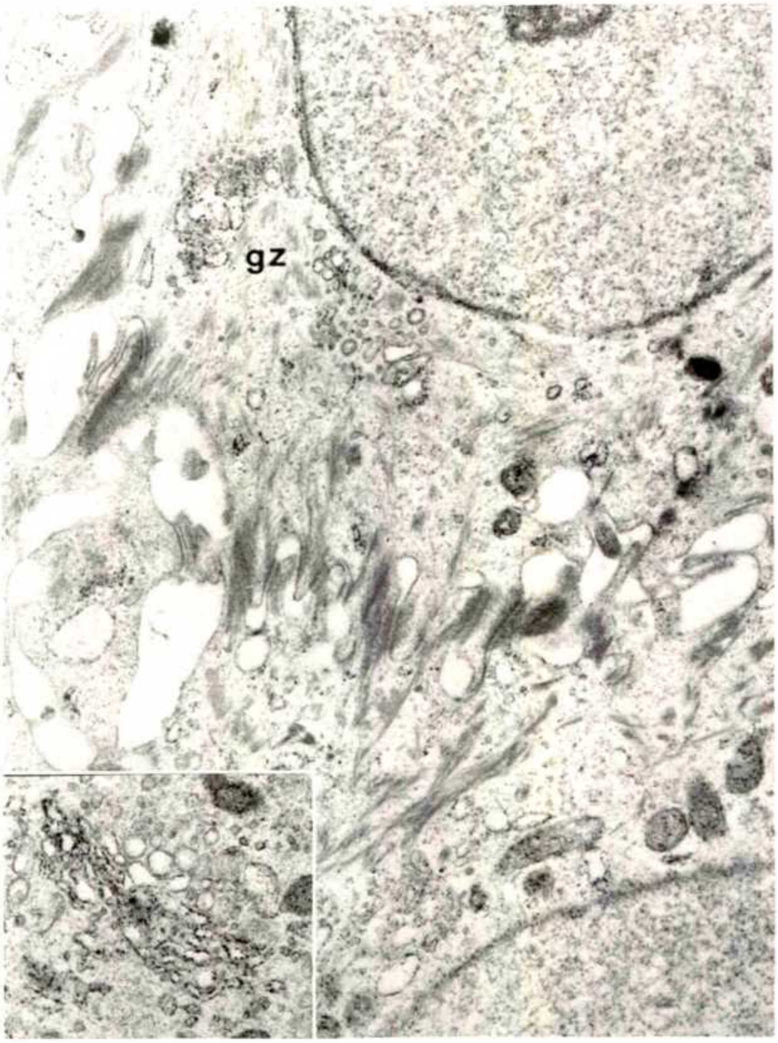


FIGURE 2. Normal keratinocyte. The fine granular reaction product labels most cytoplasmic membrane profiles, particularly the Golgi zone (gz). See also insert. The cell surface is free from labelling (Magnification × 24,000, insert × 28,000).

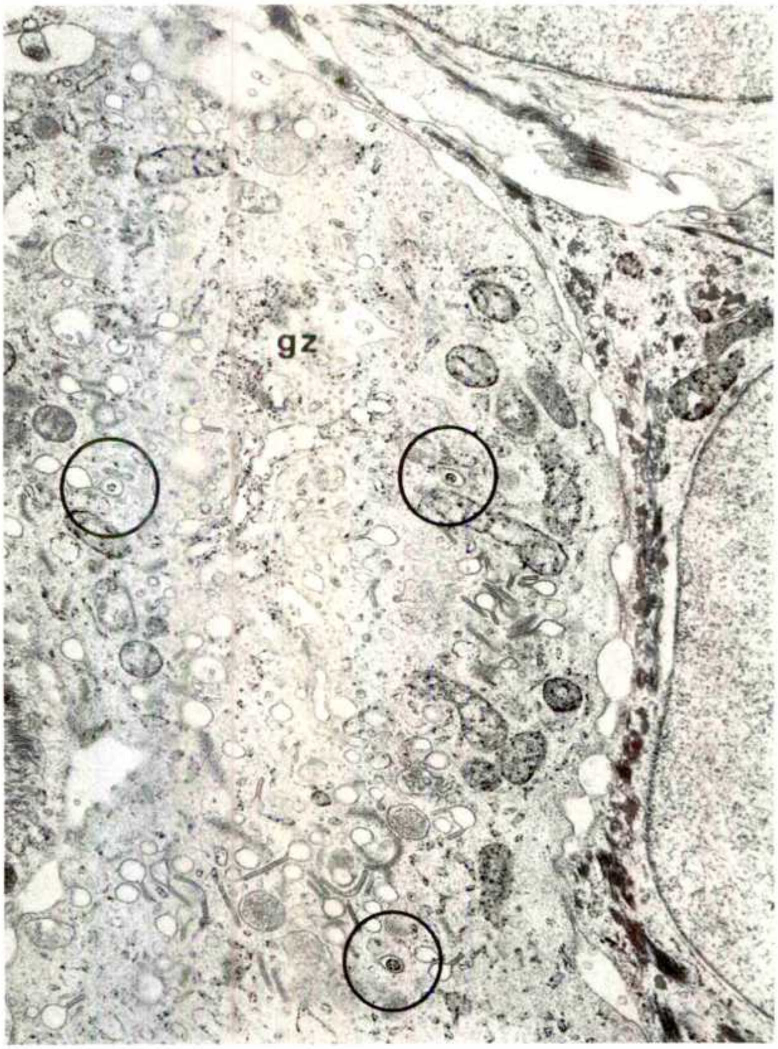


FIGURE 3. Langerhans cells show similar labelling of their cytomembranes (Golgi zone, gz). The Langerhans cell granules remain unstained. In their saccular part, however, roundish corpuscular precipitates are seen regularly (Magnification × 24,000).

the reaction varied from cell to cell and seemed to be correlated to the amount of cytoplasmic organelles. For this reason dermal cells were often more stained than the epidermal (Fig. 5). Similarly, in the epidermis the basal keratinocytes contained more electron dense material than the keratinocytes in the upper Malpighian layers.

Control preparations revealed that the precipitates in mitochondria, in melanosomes and in the nuclear envelopes were non-specific. They were also present in the controls without cAMP, without snake venom and even after incubation in a medium which contained lead nitrate only. Particularly the mitochondria and melanosomes had a high affinity to lead salts. On the other hand, the labelling of the ergastoplasm and the Golgi zone was reduced in most specimens when exogeneous nucleotidase or

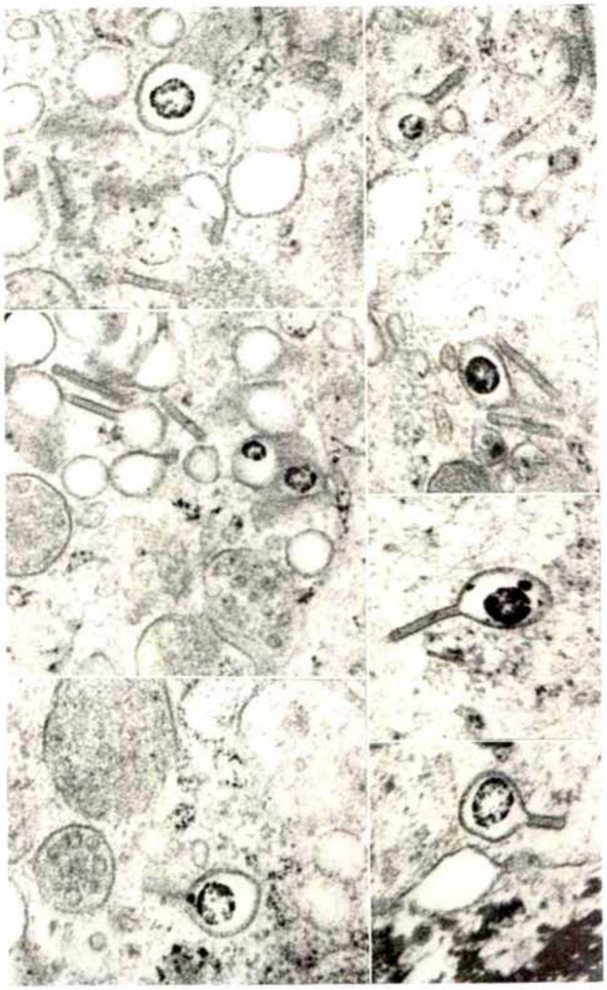


FIGURE 4. Higher magnifications of Langerhans cell granules show the corpuscular precipitates, which are always solitary and strongly osmiophilic in their periphery (Magnification × 57,000).

the substrate were omitted from the incubation medium. Also the addition of theophylline in a concentration of 200 mM seemed to decrease the lead salt precipitates on the endoplasmic and the Golgi membranes (Fig. 6).

DISCUSSION

The interpretation of cytochemical results in demonstrating PDE activity in tissue may be subject to criticism:

(a) Biochemical studies have revealed that PDE is not a uniform enzyme but probably a rather complex enzyme group. Differential centrifugation of brain homogenates showed that in contrast to the adenyl cyclase activity, which was mostly bound to the particulate fraction, the main part of PDE

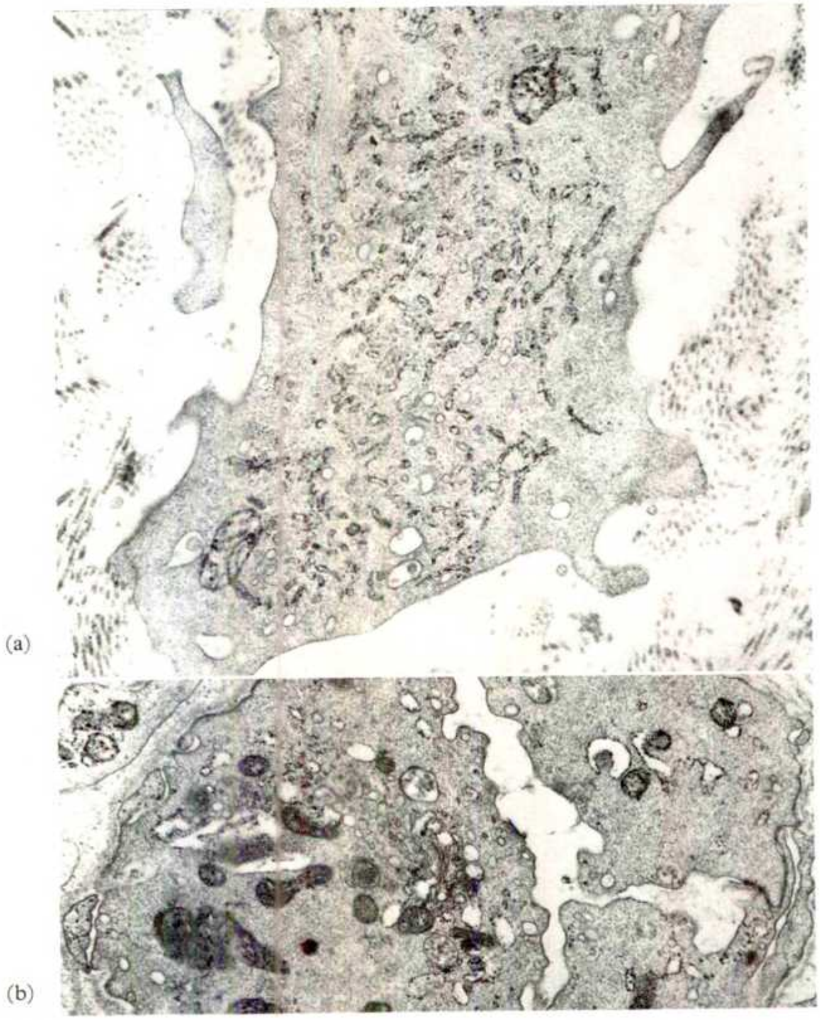


FIGURE 5. Dermal cells rich in ergastoplasm and endothelial cells show marked labelling of their membranes; in all cases the cell surfaces remain unstained (Magnification (a) × 21,000, (b) × 18, 000).

activity was found in the soluble fraction (Robertis, Lores Arnaiz & Alberici, 1967; Weiss & Costa, 1968). With cytochemical methods, therefore, the particulate membrane bound enzyme fraction may be preferentially demonstrated.

(b) Furthermore, PDE exists in a variety of isoenzymic forms, which may occur simultaneously in a particular tissue and which show a different behaviour to drugs (Moulin & Schultz, 1975). The skin of adult albino mice contains two closely related enzymes with different affinities for cAMP (Mier & Urselmann, 1972). It is possible, therefore, that the reaction product in our investigation was produced by several enzymes.

(c) Finally, the significance of this cytochemical reaction is still not clear because of the various factors which may influence the reaction product. For example, the intracellular cAMP concentration and the activity of endogeneous 5′-nucleotidase are also determining factors and may alter the final product.

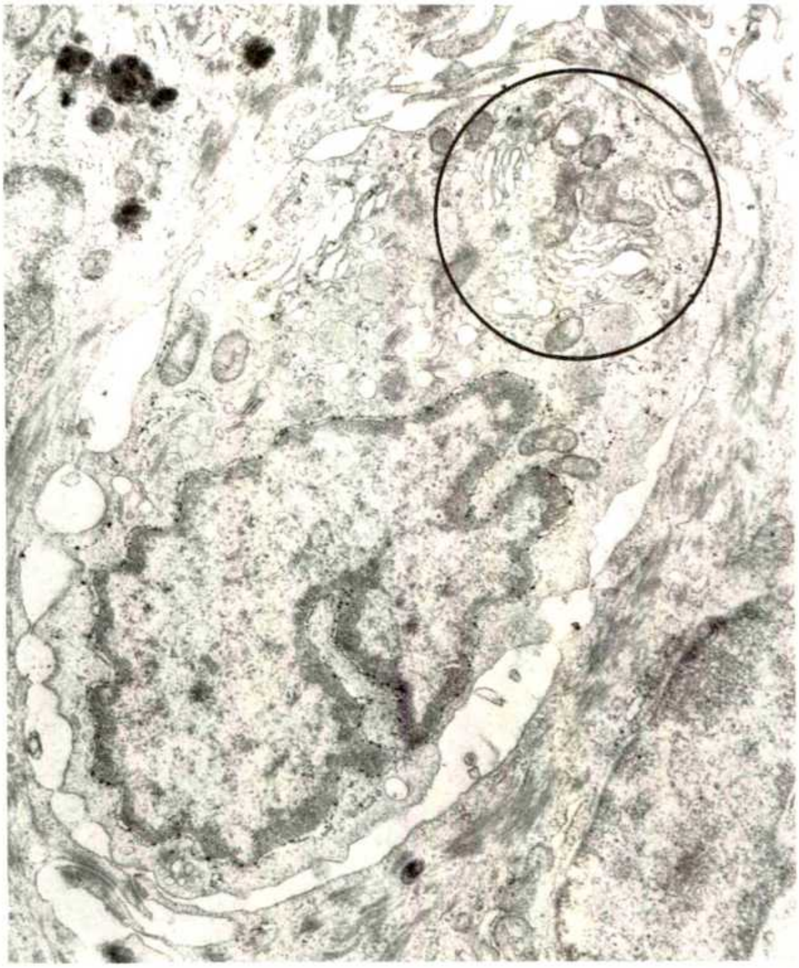


FIGURE 6. Control preparation with addition of 200 mM theophylline shows some cytoplasmic precipitates particularly on the perinuclear membrane; however, the labelling of the Golgi zone (encircled) is clearly reduced (Magnification × 21,000).

The relatively low amount of enzyme activity remaining in tissue rendered it difficult to evaluate the results. In our investigations, however, we could not confirm in human skin the observation of King *et al.* (1974), who reported that in rat skin the PDE activity was localized at the outer cell membrane. The phosphodiesterase activity, if any, was here always localized *in* the cells, namely at the cytomembranes of the endoplasmic reticulum and the Golgi zone and not on the cell surfaces. The question arises whether the formalin-containing fixative used here may inhibit PDE activity at the cell surface exclusively and/or whether the PDE pattern is different in rat and human skin. For instance, in human epidermis histamine, which is thought to affect the cAMP cascade, is a strong mitotic inhibitor. On the other hand, in the same concentration it is a stimulator of mitosis in rat skin (Flaxman & Harper, 1975). According to this, the enzymes involved in cAMP degradation and their localization may differ in human and in rat epidermis. In any case, it seems likely that the intracellular control of cAMP concentration in human skin is independent from β-receptors of the cell surface, which we showed to be changed in psoriatic epidermis (Mahrle & Orfanos, 1974; Orfanos & Mahrle, 1976).

In conclusion, our findings did not reveal any differences between normal and psoriatic skin with this cytochemical method either in the cytochemical pattern or in the intensity of the reaction under the same conditions. On the basis of these findings we assume that the cytoplasmic part of cAMP-cascade is intact in psoriatic keratinocytes, in contrast to its membrane-bound part.

ACKNOWLEDGMENT

This study was supported by the Deutsche Forschung.

REFERENCES

FLAXMAN, B.A. & HARPER, R.A. (1975) *In vitro* analysis of the control of keratinocyte proliferation in human epidermis by physiologic and pharmacologic agents. *Journal of Investigative Dermatology*, **65**, 52.

HÄRKÖNEN, M., HOPSU-HAVU, V.K. & RAIJ, K. (1974) Cyclic adenosine monophosphate, adenyl cyclase and cyclic nucleotide phosphodiesterase in psoriatic epidermis. With a comment on urinary cAMP. *Acta dermato-venerologica*, **54**, 13.

KING, L.E., FLORENDO, N.T., SOLOMON, S.S. & HASHIMOTO, K. (1974) Cyclic 3′,5′-nucleotide phosphodiesterase. I. Histochemical localization in rat skin. *Journal of Investigative Dermatology*, **62**, 485.

MAHRLE, G. & ORFANOS, C.E. (1974) Glykokalyx und Membranenzyme als epidermaler Steuermchanisemus. Veränderungen am membrangebundenen Rezeptorsystem der Adenylcyclase-cAMP-Kaskade psoriatischer Keratinozyten und ihre Bedeutung für die Atiopathogenese der Psoriasis. 30. Tagung der Deutschen Dermatologischen Gesellschaft vom 10–14 Sept. 1974 in Graz. *Archiv für Dermatologische Forschung*, 1976, in press.

MAHRLE, G. & ORFANOS, C.E. (1975) β-adrenerge Stimulation membrangebundener Adenylcyclase in normaler Epidermis und mangelnde Stimulierbarkeit bei Psoriasis. *Archiv für Dermatologische Forschung*, **253**, 195.

MIER, P.D. & URSELMANN, E. (1972) Adenosine 3′,5′-cyclic monophosphate phosphodiesterase in skin. I. Measurement and properties. *British Journal of Dermatology*, **86**, 141.

MOULIN, A.DU & SCHULTZ, J. (1975) Effect of some phosphodiesterase inhibitors on two different preparations of adenosine 3′,5′-monophosphate phosphodiesterase. *Experimentia*, **31**, 883.

ORFANOS, C.E. & MAHRLE, G. (1976) Membrandefekt als Basis der gestörten Wachstumregulation bei Psoriasis. *Dermatologica*, **151**, 199.

ROBERTIS, E. DE, LORES ARNAIZ, G.R.DE & ALBERICI, M. (1967) Subcellular distribution of adenyl cyclase and cyclic phosphodiesterase in rat brain cortex. *Journal of Biological Chemistry*, **242**, 3487.

SHANTA, T.R., WOODS, W.D., WAITZMAN, M.B. & BOURNE, G.H. (1966) Histochemical method for localization of cyclic 3′,5′-nucleotide phosphodiesterase. *Histochemie*, **7**, 177.

VOORHEES, J.J., STAWISKI, M. & DUELL, E.A. (1973) Increased cyclic GMP and decreased cyclic AMP levels in the hyperplastic, abnormally differentiated epidermis of psoriasis. *Life Sciences*, **13**, 639.

VOORHEES, J.J., KELSEY, W., STAWISKI, M., SMITH, E., DUELL, E.A., HADDOX, M. & GOLDBERG, N. (1973) Increased cyclic GMP and decreased AMP levels in the rapidly proliferating epithelium of psoriasis. In: *The role of cyclic nucleotides in carcinogenesis* (Ed. by J. Schultz and H.G. Gratzner), p. 325. Academic Press, New York.

WEISS, B. & COSTA, E. (1968) Regional and subcellular distribution of adenyl and 3′,5′-cyclic nucleotide phosphodiesterase in brain and pineal gland. *Biochemical Pharmacology*, **17**, 2107.

British Journal of Dermatology (1976) **95**, 599.

Periodic acid-Schiff (PAS) staining for glycogen in clinically normal psoriatic and non-psoriatic skin

LESLIE STANKLER AND FREDERICK WALKER*

Department of Dermatology, Aberdeen Royal Infirmary, Aberdeen, and * Department of Pathology, University of Leicester, Leicester

Accepted for publication 7 June 1976

SUMMARY

PAS staining was used to assess the glycogen content of clinically normal forearm epidermis in 32 patients with active psoriasis, 30 patients with inactive psoriasis and 40 non-psoriatic subjects. Glycogen accumulation was greater in patients with active than in those with inactive psoriasis. The inactive psoriatics showed significantly less glycogen accumulation than the controls.

In psoriatic patients glycogen is increased in affected epidermis (Halprin & Ohkawara, 1966). The reason for glycogen accumulation in human epidermis is still debated (Halprin, 1975). Montagna, Chase & Hamilton (1951) were of the opinion that it is related to defective keratinization, a view which has been questioned (Braun-Falco, 1958). Mier & Sutorius (1972) held the view that glycogen deposition is related to increase in metabolic activity but Braun-Falco (1958) stated that increased amounts of glycogen may occur in atrophic epidermis while glycogen may be absent in an epidermis which is hyperplastic. Voorhees *et al.* (1972) and Voorhees & Mier (1974) argued that in psoriatic epidermis both glycogen accumulation and accelerated cell division result from reduced activity of cyclic AMP. They stressed that glycogen accumulation is not directly related to an accelerated cell cycle but acts as an indicator of metabolic activity. Recent studies (Härkonën, Hopsu-Havu & Raij, 1974; Yoshikawa *et al.*, 1975) have failed to demonstrate reduced levels of cyclic AMP in the psoriatic plaque and it is not certain how cyclic nucleotides act to control cell proliferation and differentiation (Halprin *et al.*, 1975).

In an attempt to see whether glycogen accumulation was increased in the uninvolved as well as the involved skin of the psoriatic patient the following investigation was carried out.

PATIENTS AND METHODS

The investigations were carried out with the informed consent of the patients. Two groups of patients took part in the study. There was a group of 62 patients with psoriasis, in 30 of whom the disease was

---

Reprints requests to: Dr L. Stankler, Department of Dermatology, Aberdeen Royal Infirmary, Aberdeen, Scotland.

inactive (no fresh lesions in the last 3 months); 14 had mildly active psoriasis (a few fresh lesions); and 18 very active (many fresh lesions). Forty-two non-psoriatic individuals, with no known family history of psoriasis, acted as a control group. Three millimetre punch biopsies were taken from unaffected skin of the front of the forearm and the material was fixed in formalin, embedded in paraffin wax, ribboned, stained with PAS and graded as follows: No PAS positive granules anywhere in the epidermis except immediately related to hair follicles (−); sparsely scattered PAS positive granules in the prickle cell layer in addition to that around hair follicles in serial sections (+); widely scattered PAS positive granules in the prickle cell layer in addition to that around hair follicles (++). Absence of staining with PAS after diastase demonstrated that the material was glycogen. There was complete correlation in grading between two independent observers who examined the sections 'blind'.

RESULTS

The amount of PAS positive material present in clinically normal psoriatic and non-psoriatic epidermis is shown in Table 1. There was evidence of PAS positive material in the epidermis of 13 of 42 non-psoriatic subjects which was not significantly different from its presence in the epidermis of 14 of a total of 62 psoriatic patients, but was significantly higher than an incidence of 1 in 30 patients with inactive psoriasis ($P<0\cdot01$, $\chi^2 = 6\cdot8$, d.f. $= 1$). Seven of the 32 patients with active disease had widely distributed PAS positive material in the epidermis compared with none of 30 patients when the disease was inactive ($P<0\cdot01$). Widely distributed PAS positive material was seen only in patients with very active psoriasis.

TABLE 1. Comparison of PAS staining of unaffected and non-psoriatic epidermis

	No.	−	+	++
Active psoriatics	32	19	6	7
Inactive psoriatics	30	29	1	0
Controls	42	29	11	2

−, No PAS+ve material; +, PAS+ve material sparsely distributed; ++, PAS+ve material widely distributed.

DISCUSSION

In the present investigation the skin was taken from the front of the forearm as this site is usually free from psoriasis. There was a significant increase in glycogen in unaffected epidermis of patients with active compared with inactive stages of the disease. Although the reason for glycogen accumulation is disputed the evidence suggests that the difference in glycogen content may be related to the changes in metabolic activity of unaffected psoriatic epidermis in active and quiescent stages of the disease. This view is supported by the findings of a fall in the vitamin B12 content of unaffected psoriatic epidermis when the disease becomes active (Stankler, 1969).

An unexpected finding was reduced levels of glycogen in unaffected epidermis in patients with inactive psoriasis compared with those in non-psoriatic epidermis. These reduced glycogen levels might be due to lack of formation (possibly due to the increased activity of a metabolic inhibitor) or to increased breakdown (Halprin, 1975). It would be interesting to measure the cyclic AMP levels of unaffected psoriatic epidermis in active and inactive stages of the disease.

ACKNOWLEDGMENTS

We would like to thank Professor J.A.Milne, Dermatologist, Glasgow, Scotland and Dr S.J.Comaish, Dermatologist, Newcastle, England, for their helpful suggestions.

REFERENCES

BRAUN-FALCO, O. (1958) The histochemistry of psoriasis. *Annals of the New York Academy of Sciences*, **75**, 936.

HALPRIN, K.M. (1975) Personal communication.

HALPRIN, K.M. & OHKAWARA, A. (1966) Carbohydrate metabolism in psoriasis: An enzymatic study. *Journal of Investigative Dermatology*, **46**, 43.

HALPRIN, K.M., ADACHI, K., YOSHIKAWA, K. *et al.* (1975) Cyclic AMP and psoriasis. *Journal of Investigative Dermatology*, **65**, 170.

HÄRKONEN, M., HOPSU-HAVU, V.K. & RAIJ, K. (1974) Cyclic adenosine monophosphate, adenyl cyclase and cyclic nucleotide phosphodiesterase in psoriatic epidermis with a comment on urinary cAMP. *Acta dermato-venereologica*, **54**, 13.

MIER, P.D. & SUTORIUS, A.H.M. (1972) The control of glycogen in skin phosphorylase B kinase and phosphorylase A kinase. *British Journal of Dermatology*, **86**, 49.

MONTAGNA, W., CHASE, W.B. & HAMILTON, J.B. (1951) The distribution of glycogen and lipids in human skin. *Journal of Investigative Dermatology*, **17**, 147.

STANKLER, L. (1969) The vitamin B_{12} levels in psoriatic skin and serum. *British Journal of Dermatology*, **81**, 911.

VOORHEES, J.J., DUELL, E.A., BASS, L.J. *et al.* (1972) Decreased cyclic AMP in the epidermis of lesions of psoriasis. *Archives of Dermatology*, **105**, 695.

VOORHEES, J.J. & MIER, P.D. (1974) The epidermis and cyclic AMP. *British Journal of Dermatology*, **90**, 223.

YOSHIKAWA, K., ADACHI, K., HALPRIN, K.M. & LEVINE, V. (1975) Is the cyclic AMP in psoriatic epidermis low? *British Journal of Dermatology*, **93**, 253.

British Journal of Dermatology (1976) **95**, 603.

Bacterial flora in psoriasis

RAZA ALY, HOWARD I. MAIBACH AND ADRIAN MANDEL

Department of Dermatology, University of California, San Francisco, CA 94143, U.S.A.

Accepted for publication 11 May 1976

SUMMARY

The aerobic bacterial flora of psoriatic plaques, uninvolved skin and the anterior nares of forty psoriatic patients was studied. The incidence of *Staphylococcus aureus* was 30% in the anterior nares, 20% on the plaques and 13% on the uninvolved skin. *S. aureus* counts were $3 \times 10^2/\text{cm}^2$ on the plaques and $1{\cdot}5 \times 10/\text{cm}^2$ on the normal skin. The total bacterial counts were also higher on plaques ($7{\cdot}9 \times 10^3/\text{cm}^2$) than on normal skin ($3{\cdot}0 \times 10^3/\text{cm}^2$). The incidence of lipophilic diphtheroids was significantly lower on the plaques (4%) than the normal skin (30%). Eighty percent of the strains of *S. aureus* isolated from psoriatic patients were resistant to 10 units of penicillin. Because of increased desquamation, psoriatic skin is a public health hazard.

Most psoriatic patients do not have clinical pyoderma. Their lesions, however, can be colonized with *Staphylococcus aureus* and become a source of hospital cross-infection. Payne (1967) reported an epidemic of staphylococcal wound infection traced to an anaesthetist with psoriasis. Marples, Heaton & Kligman (1973) showed that half their psoriatic patients carried *S. aureus* on their skin, though usually in low number. Using contact plates, Noble & Savin (1968) demonstrated that 26% (9 of 34 patients) of psoriatic patients carried *S. aureus*; seven of the nine carriers were inpatients.

A major anatomical site of dissemination of *S. aureus* in a normal population is the anterior nares (White, 1961; Hallman, 1937). Depressing *S. aureus* counts in the nares reduces spread in the immediate environment (White & Smith, 1964). In previous studies of the microbiology of psoriasis the nasal carriage status of *S. aureus* has not been studied. This investigation had the following objectives:

(1) to characterize quantitatively and qualitatively the aerobic microbial flora of normal skin and psoriatic plaques; (2) to compare the carriage of *S. aureus* in the nose, non-involved and involved skin; and (3) to determine the antibiotic resistance of *S. aureus*.

MATERIALS AND METHODS

Forty outpatients with psoriasis were studied. The sites for sampling bacteria were the anterior nares, involved skin and adjacent non-involved skin. The lesions were stable plaques, mainly on the arms and legs.

Sampling methods. Samples were obtained by the detergent scrub technique (Williamson &

Kligman, 1965). An aluminium cylinder, 6·15 cm^2 in area, was held to the skin and the surface rubbed with a teflon 'policeman' in one ml of wash solution (Triton X-100 in phosphate buffer) for 1 min. Nasal flora were removed by using a calcium alginate swab which was dissolved in buffer. Appropriate 10-fold dilutions were made and cultured on blood agar plates (5% defibrinated sheep red blood agar), trypticase soy agar with Tween 80 to enhance the growth of lipophilic diphtheroids, eosin methylene blue for Gram negative rods, crystal violet agar for streptococci and Sabouraud's dextrose agar for fungi. Bacterial growth media were incubated aerobically at 35°C for 48 h. Sabouraud's agar plates were incubated at room temperature. Representative strains of different groups of bacteria were selected on colonial morphology. Gram stain was performed on selected colonies. Organisms were classified according to their response to biochemical tests, and by their growth and/or morphology on selective differential media.

RESULTS

Qualitative estimation of micro-organisms

The incidence of *S. aureus* (40 patients) was 30% in the anterior nares, 20% on the plaques and 13% on uninvolved skin (Table 1). With the exception of the plaques, the occurrence of *S. aureus* was within the range of the normal population. This higher incidence of *S. aureus* in the lesion when compared with the adjacent normal skin is not statistically significant ($P = 0·4$). More than 80% of the samples from plaques and normal skin contained coagulase negative, Gram positive cocci. The incidence of lipophilic diphtheroids was 4% in the lesions and 30% on the normal skin ($P = 0·002$). Other groups of bacteria, such as non-lipophilic diphtheroids and Gram negative rods were more frequently found in the plaques than in the normal skin. Coagulase negative Gram positive bacteria were the predominant flora in both lesions and normal skin. The composition of nasal flora was within the normal range and comparable with normal populations (Aly *et al.*, 1970).

TABLE 1. Percentage incidence of organisms in psoriatic patients (40 subjects)

Micro-organisms	Anterior nares	Plaques	Uninvolved skin
S. aureus	30	20	13
Coagulase negative Gram positive cocci	92	82	82
Non-lipophilic diphtheroids	47	30	23
Lipophilic diphtheroids	50	4	30
Streptococci	2	15	15
Gram negative rods	5	10	8

Semi-quantitative estimation of microflora

The average total aerobic counts were slightly higher in the lesions than on the normal skin ($7·9 \times 10^3$ vs $3 \times 10^3/\text{cm}^2$). The density of *S. aureus* was higher in the plaques (with an average count of $3 \times 10^2/\text{cm}^2$) than in the uninvolved skin ($1·5 \times 10^1/\text{cm}^2$) but this difference was not statistically significant ($P = 0·25$). *S. aureus* was the major component of the flora in more than 30% of the lesions. The density of lipophilic diphtheroids was $6·7 \times 10^1/\text{cm}^2$ in plaques and $1 \times 10^3/\text{cm}^2$ on normal skin ($P = 0·05$). Coagulase negative Gram positive cocci were the predominant organisms in the plaques and constituted 52% of the total aerobic bacterial flora, followed by nonlipophilic diphtheroids (43%) and *S. aureus* (4%). On normal skin coagulase negative, Gram positive cocci were also the predominant organisms (57%), but were followed by lipophilic diphtheroids (33%) and nonlipophilic diphtheroids (9%).

TABLE 2. Average microbial counts in psoriatic patients (40 subjects)

Micro-organisms	Anterior nares	Plaques*	Uninvolved skin*
S. aureus	$1{\cdot}2 \times 10^4$	$3{\cdot}0 \times 10^2$	$1{\cdot}5 \times 10^1$
Coagulase negative Gram positive cocci	$3{\cdot}3 \times 10^5$	$4{\cdot}1 \times 10^3$	$1{\cdot}7 \times 10^3$
Non-lipophilic diphtheroids	$1{\cdot}2 \times 10^5$	$3{\cdot}0 \times 10^2$	$2{\cdot}7 \times 10^2$
Lipophilic diphtheroids	$9{\cdot}1 \times 10^4$	$6{\cdot}7 \times 10^1$	$1{\cdot}0 \times 10^3$
Streptococci	$2{\cdot}0 \times 10^2$	$3{\cdot}5 \times 10^1$	$3{\cdot}8 \times 10^1$
Gram negative rods	$2{\cdot}7 \times 10^3$	$< 10^1$	$< 10^1$

* Per cm².

Antibiogram. The antibiotic resistance of *S. aureus* strains isolated from psoriatic patients was determined by the paper disc method. Eighty percent of the strains were resistant to 10 units of penicillin; all strains were sensitive to cephalothin, tetracycline, erythromycin, kanamycin, oxacillin, novobiocin and neomycin (Bauer *et al.*, 1966).

DISCUSSION

This study confirms the previous findings (Marples *et al.*, 1973; Noble & Savin, 1968) that the density of microbial flora is higher on the psoriatic plaques than on uninvolved skin. Marples demonstrated a 50% carriage rate of *S. aureus* in a psoriatic population, and Noble & Savin (1968) reported a 26% carriage rate of *S. aureus*. About half the subjects in this latter study were in-patients. In our investigation the *S. aureus* carriage was 20% in the psoriatic plaques; this could be due to the fact that our subjects were outpatients. Although the frequency of *S. aureus* in plaques was higher when compared with the adjacent normal skin, the difference was not statistically significant. The carriage rate of *S. aureus* on the skin of the normal population (without psoriasis) is less than 10% (unpublished data).

The density of *S. aureus* was much greater on the plaques ($3{\cdot}0 \times 10^2/\text{cm}^2$) than on uninvolved skin ($1{\cdot}5 \times 10^1/\text{cm}^2$). We assume that once *S. aureus* lands on a psoriatic plaque its chances of colonizing and surviving are greater than on the normal skin. With the exception of lipophilic diphtheroids, other categories of bacteria occurred with similar densities on the plaques and the normal skin. The incidence of lipophilic diphtheroids in the plaques was much lower (4%) than on normal skin (30%). In the lesions, lipophilic diphtheroids constituted less than 1% of the total flora, while on norma skin with exception of coagulase negative, Gram positive cocci, these were the predominant organisms. *S. aureus* did not constitute the major component of the flora in the plaques of the majority of the patients. Coagulase negative, Gram positive cocci were the most frequent bacteria in the lesions and normal skin. Although abnormal skin is a common factor in psoriatics and atopics, their microbial flora are quite different from each other. When comparing the microbial flora of psoriatics to that of atopic dermatitis (Leyden, Marples & Kligman, 1974; Aly *et al.*, 1976, submitted for review), the following major differences were noted:

(1) The incidence (90–100%) and density ($7{\cdot}5 \times 10^4/\text{cm}^2$) of *S. aureus* were much higher in atopic lesions than in psoriatic plaques (20% incidence and $3 \times 10^2/\text{cm}^2$ density); (2) *S. aureus* was the

predominant organism in the lesions of atopics, while coagulase negative, Gram positive cocci were the major organisms in psoriatic plaques; (3) The scarcity of lipophilic diphtheroids noted in psoriasis was even more pronounced in the lesions of atopics; (4) The nasal carriage of *S. aureus* was much higher in atopics (79%) than in psoriatic patients (30%). The nasal carriage of *S. aureus* in psoriatics is comparable to that of normal population.

Microbial studies of the psoriatic plaques suggest that the higher counts of *S. aureus* are not related to clinical infection of the lesions. Noble & Savin (1968) reported that 44% of 16 inpatients with psoriasis yielded *S. aureus* from plaques; Selwyn & Chalmers (1965) found 35% of 114 patients similarly colonized. However, they both found that outpatients with psoriasis seldom yielded *S. aureus* on their skin. On the contrary, Marples found high carriage of *S. aureus* (50%) in outpatients.

The incidence of *S. aureus* in our population (20%) and in other similar studies (26% and 50%, Noble and Marples) is not as high as in atopic dermatitis (Leyden *et al.*, 1974; Aly *et al.*, 1976, submitted for review). However, it seems that once *S. aureus* gains a foothold on psoriatic plaques, its numbers increase significantly. It is not understood why these patients do not develop frank pyoderma more frequently: they often have *S. aureus* colonization and some are immunosuppressed (Epstein & Maibach, 1965). Considering the high rate of desquamation of psoriatic skin, dispersal of *S. aureus* is a public health hazard and should not be taken lightly.

ACKNOWLEDGMENTS

The authors wish to thank Charlene Shirley and Walter Lindsay for their technical assistance. This investigation was supported by Research Grant Number AI 1085604 from the NIH and NCA2-OR665-601, NASA.

REFERENCES

Aly, R., Maibach, H.I., Shinefield, H.R. & Strauss, W. (1970) Effect of systemic antibiotics on nasal bacterial ecology in man. *Applied Microbiology*, **20**, 240.

Aly, R., Maibach, H.I. & Shinefield, H.R. (1976) Microbial flora of atopic dermatitis, submitted for publication.

Bauer, A.W., Kirby, W.W., Sherris, J.C. & Turck, M. (1966) Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, **45**, 493.

Epstein, W. & Maibach, J.I. (1965) Immunologic competence of patients with psoriasis receiving cytotoxic drug therapy. *Archives of Dermatology*, **91**, 599.

Hallman, F.A. (1937) Pathogenic staphylococci in the anterior nares: Their incidence and differentiation. *Proceedings of the Society of Experimental Biology and Medicine*, **36**, 789.

Leyden, J.J., Marples, R.R. & Kligman, A.M. (1974) *Staphylococcus aureus* in the lesions of atopic dermatitis. *British Journal of Dermatology*, **90**, 525.

Marples, R.R., Heaton, C.L. & Kligman, A.M. (1973) *Staphylococcus aureus* in psoriasis. *Archives of Dermatology*, **107**, 568.

Noble, W.C. & Savin, J.A. (1968) Carriage of *Staphylococcus aureus* in psoriasis. *British Medical Journal*, **i**, 417.

Payne, R.W. (1967) Severe outbreak of surgical sepsis due to *Staphylococcus aureus* of unusual type and origin. *British Medical Journal*, **iv**, 17.

Selwyn, S. & Chalmers, D. (1965) Dispersal of bacteria from skin lesions, a hospital hazard. *British Journal of Dermatology*, **77**, 349.

White, A. (1961) Quantitative studies on nasal carrier of staphylococci among hospitalized patients. *Journal of Clinical Investigation*, **40**, 23.

White, A. & Smith, J. (1964) Nasal reservoir as the source of extranasal staphylococci. *Proceedings of the Third International Conference on Antimicrobial Agents and Chemotherapy, Washington D.C.*, p. 679.

Williamson, P. & Kligman, A.M. (1965) A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology*, **45**, 498.

British Journal of Dermatology (1976) **95**, 607.

Effects of occlusion upon population dynamics of skin bacteria

DAVID J.BIBEL, DAVID J.LOVELL AND ROKO J.SMILJANIC

Department of Dermatology Research, Letterman Army Institute of Research, San Francisco, California U.S.A.

Accepted for publication 22 May 1976

SUMMARY

Quantitative and qualitative changes of the cutaneous aerobic bacterial flora upon 20 sites on the backs of each of three healthy subjects were examined before and after one site was occluded, using skin flora maps as a tool. Major local alterations were found to affect the carriage of micro-organisms in distant surrounding areas. *Staphylococcus epidermidis* was the most successful competitor. Furthermore, some sites appeared to act as restricted reservoirs for specific types of micro-organisms whereas other areas were less limited in their support of flora.

The composition of the cutaneous flora on a particular site is not static, but rather constantly changes with every subtle yet poorly defined alteration of the microhabitat (Williamson & Kligman, 1965; Noble & Somerville, 1974; Kloos & Musselwhite, 1975). In addition, areas just a few centimetres apart are known to have different populations and even different kinds of micro-organisms within the *Micrococcaceae* (Williamson & Kligman, 1965; Shaw, *et al.*, 1970; Bibel & Lovell, 1976). As yet no study has examined alterations in the flora on multiple adjacent sites both quantitatively and qualitatively. Data from such an investigation may be depicted as maps whose patterns offer a means of detailed analysis of skin ecology (Bibel & Lovell, 1976).

This paper describes the population dynamics of skin flora under the environmental assault of occlusion. Like the rippling effect produced by a stone dropped into a tranquil pond, a major, localized change of flora is shown to affect the carriage of bacteria in surrounding areas. Furthermore, we note that some sites seem to act as reservoirs for specific types of micro-organisms whereas other areas support a less restricted flora.

MATERIALS AND METHODS

Volunteers. Three healthy white men, 25–35 years of age, participated in this study. All used a non-deodorant soap for at least 1 month prior to the experiment. Washing during the period of study was not restricted.

Sampling site. The back was chosen as a test area because of its large expanse of similar epidermis, its restricted exposure due to protective clothing and its apparently uniform microclimate. A grid was drawn on the back of each subject with a pen whose water-soluble ink was neither toxic, germicidal nor a source of micro-organisms. The network was composed of 20 5 × 5 cm squares arranged in 4 columns across, bisected by the spine, and 5 rows down, beginning at a line connecting the depth of the axillary vaults. Part of the scapular region was included within the test zone.

Sampling method. The centre of each of the 20 squares was sampled using a sterile stainless steel well measuring 1·5 × 1·5 cm as a sampling chamber. Hence, about 9% of the area of the grid was sampled. A sterile, blunted stainless steel spatula with a 250 g weight attached to its handle was utilized to scrape the site with a uniform pressure. One ml of phosphate-buffered Triton X-100, pH

7·9 (Williamson & Kligman, 1965), was first placed in the chamber and the spatula was moved linearly for 30 cycles. The fluid was harvested and a second ml of buffer was added. Again the site was scraped for 30 cycles. A third ml served as final rinse and the pooled specimen was diluted in serial 10-fold steps for inoculation in triplicate on plates of trypticase soy agar.

Identification of flora. Only the aerobic flora was studied. The plates were incubated at 35°C under 5% CO_2 in air for 48–72 h; all colonies were counted and identified according to *Bergey's Manual of Determinative Bacteriology* (Buchanan & Gibbons, 1974) using a replica-plating procedure (Bibel, Smiljanic & Lebrun, 1975). Large and small-colony diphtheroids were simply grouped but *Micrococcaceae* were classified to the biotype level. Gram negative bacilli were identified using API strips (Analytab Products, Inc., Plainview, New York) or standard methods (Branson, 1972). We followed the procedures of Gordon, Haynes & Hor-Nay Pang (1973) to identify *Bacillus* species.

Experimental design. All sites on the back were sampled five times, the first two occasions being on successive days. After the second sampling the square located at the second column from the left and third row down (site 10, Fig. 1) was occluded for 48 h in the following manner. Two layers of sterile gauze, measuring 4 × 4 cm were placed upon the skin and moistened with 0·2 ml of sterile distilled water. Medical Adhesive B (Dow Corning Corp., Midland, Michigan) was spread around the perimeter with a sterile swab and then a 5 × 5 cm sheet of Teflon was pressed upon the site. The dressing was further secured by tape. On day 4, 48 h later, the dressing was removed and specimens of flora were obtained, the occluded site being sampled last. Additional samplings were conducted on day 5, and on day 7, 72 h after removal of the dressing. Maps were constructed for each volunteer depicting the microbial density and proportion of the kinds of micro-organisms found.

RESULTS

To orient the reader to the maps depicted in Figs 1–3, we define a grid as a unit of 20 squares and a category of grids as the listed nomenclature of columns referring to total or kinds of flora (for example, there are 5 grids representing the *S. saprophyticus* biotype 1 category). The 'total aerobic flora' key pertains only to the first grid category. Throughout this section reference is made to site numbers as shown in Fig. 1.

Normal distribution of flora. With the exception of site 10 on day 4 (after occlusion), the maps of the first three sampling periods represent normal, healthy skin. The general lack of bilateral symmetry

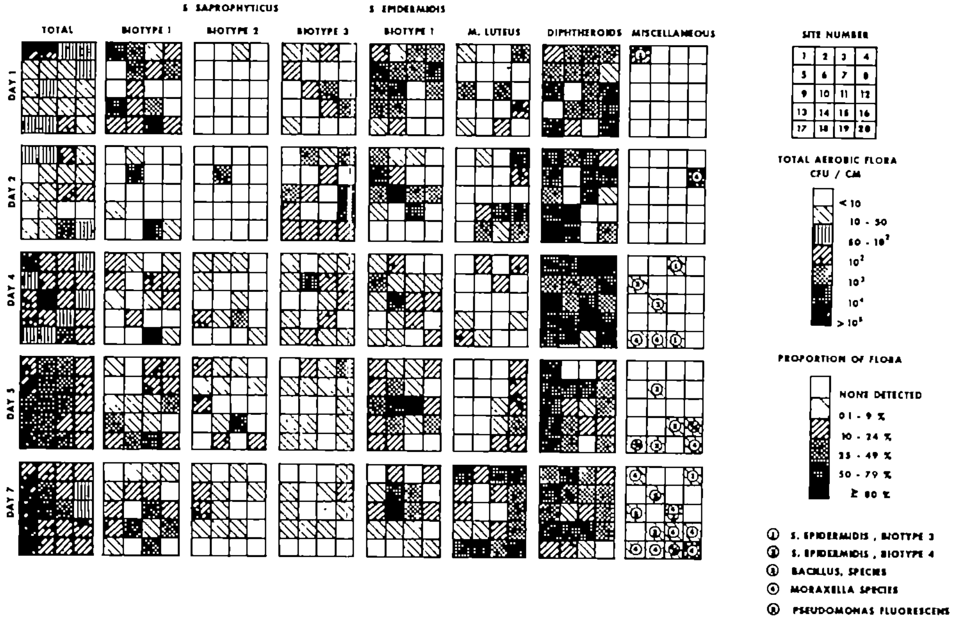


FIGURE 1.

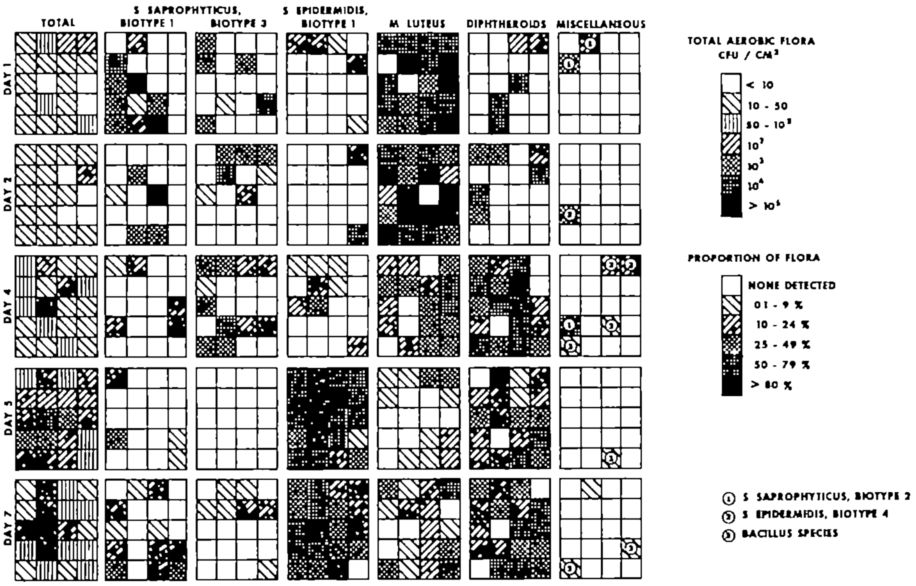


FIGURE 2.

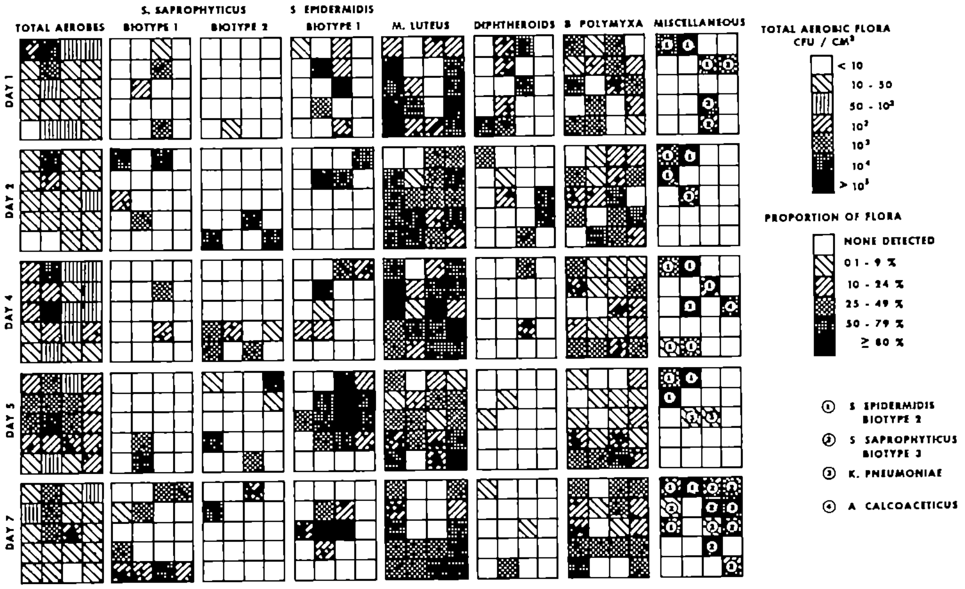


FIGURE 3.

FIGURES 1–3. Skin flora maps of the backs of three individuals. Patterns show dynamics of bacterial populations before and after occlusion. Site 10 was occluded between Days 2 and 4. Fig. 1. Subject 1. Fig. 2. Subject 2. Fig. 3. Subject 3.

or of consistent patterns is readily apparent. Multiplying the 9 symmetrical pairs of normal sites, the 7 or 8 categories of grids, and these 3 sampling days, an average proportion of anatomically symmetrical paired sites with flora was determined for each of the 3 subjects. The proportions were 12·1%, 9·8%, and 11·2%, with combined range of 7·4–15·0%. Bilaterally symmetrical distributions were limited to only one grid category, and no two anatomically symmetrical sites were both quantitatively or qualitatively alike. Since the figures display arbitrarily grouped data, we should note that the chances of perfect symmetry would be remote if original calculated amounts are considered. Left- or right-handed dominance of distribution on anatomically symmetrical sites was not found on the total flora grid (χ^2, $P>0{\cdot}05$ for individuals or pooled data).

Careful inspection, however, determined that the patterns for these 3 days were not random. Examining the total-flora grid, we found that the top 2 rows of sites generally carried higher numbers of flora than the lower two rows of sites (*t*-test, $P<0{\cdot}01$ for pooled data, $P>0{\cdot}05$ for subject 1; $P<0{\cdot}05$ for subject 2; $P<0{\cdot}01$ for subject 3), a reflection of the more nutritious substrate of the scapular areas. This was demonstrated not only by the preponderance of flora on one particular site but also by the number of sites with large populations (Table 1).

Certain sites of individuals were noteworthy for the numerical stability in their carriage of flora. For instance, in the total-flora grid, sites 6, 11, 13, 17, and 18 of subject 2 (Fig. 2) and sites 2, 7, 13, and 20 of subject 3 (Fig. 3) remained unchanged over the 4-day period. Other sites, such as site 19 of subject 1 (Fig. 1) and sites 2 and 6 of subject 3 (Fig. 3), when compared to surrounding areas, were distinctly higher in total flora during this period. On the other hand, sites 12 and 16 of subject 2 (Fig. 2) supported little flora. This relative consistency in localized carriage of micro-organisms during the experimental period suggested the concept of reservoirs.

Analysis of qualitative data tended to support this hypothesis. Subject 1 offered a good example. Site 19, possessing the largest amount of flora, also was dominated by *Staphylococcus saprophyticus* biotype 1, although the organism was absent on nearby site 16. *Staphylococcus epidermidis* biotype 1 seemed to focus on sites 5, 10, and 12 and *Micrococcus luteus* resided on sites 4 and 16. Similarly, the other volunteers showed foci of flora, such as *M. luteus* on site 12 of subject 2, and *S. epidermidis* biotype 1 on site 6 and *M. luteus* on sites 5, 8 and 16 of subject 3. These reservoirs seemed to dominate adjacent sites, and territories could be traced. The territories of different micro-organisms overlapped. Some areas lacking influence by a major reservoir were more susceptible to flux. Reservoir locations were different on each individual.

Environmental assault. What happens when the cutaneous ecosystem is subjected to a localized stress of major magnitude, such as occlusion? All volunteers initially yielded greater than 10^5 colony-forming units at site 10 upon removal of the dressing. In one case *Klebsiella pneumoniae* was found, whereas in the other two subjects diphtheroids and *S. epidermidis* biotype 1 shared the site. By the following day the flora was reduced by tenfold and it was clear that the flora of the new reservoirs spread to both adjacent and distant sites, tending toward the left and lower regions of the test zone.

TABLE 1. Relationship of sampling site location (see Fig. 1) to total aerobic flora, colony-forming units/cm^2. Higher counts in upper sites probably reflect influence of sebaceous nutrients

	Subject 1		Subject 2		Subject 3		Pooled data	
cfu/cm^2	Sites 1–8	Sites 13–20	Sites 1–8	Sites 13–20	Sites 1–8	Sites 13–20	Sites 1–8	Sites 13–20
$<10^2$	5·0*(62·5)†	5·7(70·8)	6·3(78·8)	8·0(100)	5·0(62·5)	7·3(91·7)	5·4(68·1)	7·0(87·5)
$>10^2$	3·0(37·5)	2·3(29·2)	1·7(21·2)	0(0)	3·0(37·5)	0·7(8·3)	2·6(31·9)	1·0(12·5)

* Average tally of sites for 3 days from a total of 8 sites; † percentage in parentheses.

Indeed, the influence went beyond the boundary. Although *K. pneumoniae* spilled over to another site, it was *S. epidermidis* biotype 1 on all 3 volunteers which quickly seized territory, even though it was at first in the minority. This is particularly observed in subject 2 (Fig. 2). By the last sampling day, the test zone was returning to normal with only site 10 and its immediate neighbours harbouring the staphylococcus in high numbers and proportions. *K. pneumoniae* had disappeared.

During this ordeal diphtheroids and the *Bacillus* of subject 3 remained essentially unaltered. There was an insufficient incidence of other staphylococcal species to judge any significant effect on subjects 2 and 3, but *S. saprophyticus* biotype 1 on subject 1 did not show any major or distinct change in distribution. *M. luteus*, however, seemed to have suffered by the influx of *S. epidermidis*. In all volunteers, but especially on subject 2, the micrococcus was forced to the periphery; however, it soon regained its domain, first spreading into the test zone from right-side reservoirs. These sources of bacteria were discernible during the entire period of study. As final notes, we should add that the skin remained normal in appearance, except at site 10 where a miliaria-like rash developed on subjects 1 and 2. Pruritus and erythema subsided in 3–4 days. *B. polymyxa* was subsequently lost from subject 3 after 2 months.

DISCUSSION

Human skin forms a microbial ecosystem and has yet to undergo a rigorous quantitative ecological analysis. Our study may be one step towards this goal, since maps have been effectively utilized in the examination of larger ecosystems (Pielou, 1969; Poole, 1974). Our maps clearly show the variation in microbial inhabitation which can occur on essentially similar epidermis. Indeed, anatomically symmetrical sites carry different levels and kinds of micro-organisms (Bibel & Lovell, 1976; Shaw *et al.*, 1970).

Our data indicate that under normal conditions for at least a week some areas of the skin can act as reservoirs for certain bacteria or consistently support high or low populations of flora. These areas thus constitute different habitats than sites a few centimetres away. Possible explanations for this persistent local variation include the number and arrangement of hair follicles and sweat ducts within uniformly measured sites, the varying availability of nutrients during keratinization of epithelium, and subtle gradients in temperature and in emission of carbon dioxide and water vapour. These or similar factors are probably instrumental in the selection of specific micro-organisms able to reside on the skin (Alexander, 1971).

The size and physical nature of cutaneous microbial reservoirs is unknown. Our sampling area was about 2 cm^2, a massive territory from the view of a microbe. Most aerobic flora seem to dwell at the openings of hair follicles (Montes & Wilborn, 1969; Bibel, unpublished observations by scanning electron microscopy); however, Puhvel, Reisner & Amirian (1975) suggest that aerobes on the back reside on the stratum corneum surface. Although microcolonies of 2–10,000 units have been calculated (Somerville & Noble, 1973), their analysis, based on total flora and the number of colonies on contact plates, did not consider qualitative data nor the number of follicles within the sampling site. The size and number of microcolonies within or adjacent to follicles remain obscure.

If reservoirs are associated with hair follicles, they probably are not limited to a single or a discrete collection of these follicles. As suggested by our maps, specific micro-organisms within the reservoir area may be found in increasing proportions toward a central follicular zone which provides the best conditions for growth and survival. A corollary is that multiple kinds of micro-organisms share the peripheral follicles; only the central follicles of the reservoir are restricted in types of flora.

Although the skin flora was sensitive to minor alterations of the micro-environment, the ecosystem generally was resilient, and quickly returned to its normal state after major fluctuations. Our previous study of occlusion (Bibel & Lebrun, 1975) focused on the occluded site itself. We had observed that *Enterobacteriaceae*, normally rare on exposed skin, flourished for a short while and that about a week was required before the site's ecological profile returned to its initial conditions. In the present report

Klebsiella was again found on the occluded site, but we did not detect any spreading to distant areas. Regrettably, we did not follow the changes in flora beyond the third day after the removal of dressings. We did not anticipate the large influence that the altered site had on surrounding areas. The population in the occluded site remained elevated at this time but the neighbouring areas were clearly regaining their former ecological balance. It is likely that complete recovery would have been achieved in 2 or 3 additional days.

The spreading of different flora following the removal of occlusive dressings did not cause a replacement of reservoir flora. Under ecological theory (Alexander, 1971), this is not because the site was already physically occupied, but because the niche was already satisfied and the foreign bacteria were unable to compete and survive. Although other kinds of flora were plentiful on the occluded site, we found *S. epidermidis* in high proportions on distant sites. Spreading was achieved by mechanical means, such as by washing (one subject refrained from showering during the experiment) and by retention and distribution of desquamated rafts by clothing (Noble, 1975). The relatively long-term and increased presence of the staphylococcus at these peripheral sites is indicative of the organism's superior ability to survive, to resist better the host and microbial antagonistic or competitive agents, and to match more closely the niche for that particular site.

Ecology is a science of subtleties and interactions which requires a complete overview. Most experiments in cutaneous microbiology have dealt with small parts of the ecosystem with little regard for the other components. Hopefully, future quantitative studies utilizing skin maps and other such tools can bring together diverse information. By treating human skin as a biological and ecological system, the role of normal flora in cutaneous health, infection and immunity may be better understood.

REFERENCES

Alexander, M. (1971) *Microbial Ecology*. John Wiley, New York.

Bibel, D.J. & Lebrun, J.R. (1975) Changes in cutaneous flora after wet occlusion. *Canadian Journal of Microbiology*, **21**, 496.

Bibel, D.J., Smiljanic, R.J. & Lebrun, J.R. (1975) A replica-plating method for the identification of Micrococcaceae. *Canadian Journal of Microbiology*, **21**, 1976.

Bibel, D.J. & Lovell, D.J. (1976) Skin flora maps: a tool in the study of cutaneous ecology. *Journal of Investigative Dermatology*, **66**, 265.

Branson, D. (1972) *Methods in Clinical Bacteriology. A Manual of Tests and Procedures*. Charles C.Thomas, Springfield, Illinois.

Buchanan, R.E. & Gibbons, N.E. (ed.) (1974) *Bergey's Manual of Determinative Bacteriology*, 8th edn. William & Wilkins, Baltimore.

Gordon, R.E., Haynes, W.C., & Hor-Nay Pang, C. (1973) *The Genus Bacillus. Agriculture Handbook* No. 427. United States Department of Agriculture, Washington, D.C.

Kloos, W.E., & Musselwhite, M.S. (1975) Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Applied Microbiology*, **30**, 381.

Montes, L.F. & Wilborn, W.H. (1969) Location of bacterial skin flora. *British Journal of Dermatology*, **81**, Suppl. 1, 23.

Noble, W.C. (1975) Dispersal of skin micro-organisms. *British Journal of Dermatology*, **93**, 477.

Noble, W.C. & Somerville, D.A. (1974) *Microbiology of Human Skin*, p. 329. W.B.Saunders, London.

Pielou E.C. (1969) *An Introduction to Mathematical Ecology*. Wiley-Interscience, New York.

Poole, R.W. (1974) *An Introduction to Quantitative Ecology*. McGraw-Hill, New York.

Puhvel, S.M., Reisner, R.M. & Amirian, D.A. (1975) Quantification of bacteria in isolated psilosebaceous follicles in normal skin. *Journal of Investigative Dermatology*, **65**, 525.

Shaw, C.M., Smith, J.A., McBride, M.E. & Duncan, W.C. (1970) An evaluation of techniques for sampling skin flora. *Journal of Investigative Dermatology*, **54**, 160.

Somerville, D.A. & Noble, W.C. (1973) Microcolony size of microbes on human skin. *Journal of Medical Microbiology*, **6**, 323.

Williamson, P. & Kligman, A.M. (1965) A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology*, **45**, 498.

British Journal of Dermatology (1976) **95**, 613.

Airborne contact dermatitis from Compositae oleoresins simulating photodermatitis

NIELS HJORTH, JYTTE ROED-PETERSEN AND KRISTIAN THOMSEN

Department of Dermatology, Gentofte Hospital, 2900 Hellerup, Denmark, and Department of Dermatology, Finsen Institute, Copenhagen, Denmark

Accepted for publication 9 April 1976

SUMMARY

American ragweed (*Ambrosia*) dermatitis has an airborne pattern and is caused by lipid soluble oleoresins of pollens. A similar weed dermatitis has been described in India and Australia. The weeds involved were all of the Compositae family and contain sesquiterpene lactones with one common antigenic determinant.

This study reports seven Danish patients treated for many years under the diagnosis of photodermatitis, who eventually proved to suffer from Compositae oleoresin dermatitis.

Probably, Compositae dermatitis is a world-wide disease, although often misdiagnosed as has happened in Scandinavia.

Compositae is the second largest plant family and has a world-wide distribution. Members of this family are common weeds as well as popular garden and indoor plants.

Reports on contact dermatitis from Compositae species are numerous. Mitchell (1969) exhaustively reviewed the literature of Compositae dermatitis and listed all species involved. Species within only 3 of the 12 Compositae tribes account for most cases. The allergens responsible have been isolated and identified as sesquiterpene lactones (Mitchell *et al.*, 1970, 1971a) (Fig. 1).

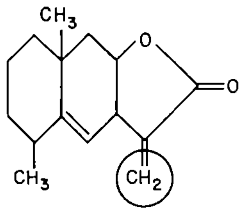


FIGURE 1. Alantolactone. Essential antigenic determinant is circled (Mitchell, 1970).

The Compositae can be classified into those with large pollen grains which are insect-pollinated and those with wind-borne pollen. Patients sensitized primarily by contact with plants like chrysanthemum may subsequently react to relatively small amounts of wind-borne pollens (Rook, 1962).

As will appear, however, there are reasons to suppose it is not so much the pollens as other organs of the plants which are implicated in the causation of the type of dermatitis here described.

American ragweeds (*Ambrosia*) cause a dermatitis of airborne pattern (Epstein, 1960), i.e. a dermatitis involving all exposed areas. Two types of dermatitis have been described: (1) Seasonal aggravation of atopic dermatitis in adolescents with a positive reaction to intradermal tests with aqueous extracts of ragweed pollen; this is quite rare just as summer aggravation of atopic dermatitis in grass pollen sensitive patients is uncommon in Denmark. (2) Ragweed contact dermatitis has the following characteristics: it is a chronic infiltrated dry dermatitis resembling atopic dermatitis, limited to exposed areas. Initially it is confined to the ragweed pollen season and it occurs preponderantly in male farmers between the ages of 40 and 65 years. Many of those affected have a personal or family history of atopy. These patients have a positive patch test to the lipid soluble fractions of ragweed pollen and also show cross sensitivity to other Compositae. One case of airborne pattern dermatitis caused by an imported Compositae species (*Xanthium*) has been reported from Britain (Rook, 1962).

A similar dermatitis caused by *Parthenium hysterophorus*, imported with American grain to Poona (India), has affected hundreds of patients who under the local economic circumstances cannot be adequately treated. Many die from a subsequent erythroderma (Lonkar, Mitchell & Calnan, 1974).

Australian bush dermatitis has all the characteristics of the American ragweed dermatitis and is caused by Compositae growing in the dry Australian bush (Burry *et al.*, 1973).

Causative factors of the dermatitis. Windborne pollen seems to be the obvious cause of ragweed dermatitis (Epstein, 1972). But many of the Compositae causing a similar pattern of dermatitis are not wind-pollinated. So Howell (1971) assumes that weed dermatitis is caused by other organs of the plant.

New facts to pinpoint the causative parts of the plants have recently been brought up by Rodriquez *et al.* (1976). In a study of *Parthenium hysterophorus* they found the highest concentration of the allergenic sesquiterpene lactones parthenin and ambrosin in the capitulum and leaves. By scanning electron microscopy trichomes were detected on the leaf surface, and examination of the trichome exudates established the presence of parthenin and ambrosin. Similar sesquiterpene-producing glands are also found in species of several other Compositae tribes. Excretions from ruptured glands as well as wind disseminated fragmented dry leaves may be the common cause of airborne contact dermatitis from Compositae which are not wind pollinated.

We wish to present a series of Danish patients with a seasonal dermatitis of light exposed areas. They had all been assumed to suffer from photodermatitis but, eventually—sometimes after a period of years—the diagnosis had to be revised to Compositae dermatitis.

MATERIALS AND METHODS

This study comprises selected patients, patch tested with Compositae oleoresins because of a dermatitis of light exposed areas. All patients had initially been patch tested with a series of standard patch tests as described by the International Contact Dermatitis Research Group. Age, sex and clinical history are shown in Table 1.

Oleoresins in petrolatum for closed patch testing were supplied on an experimental basis by the courtesy of Dr Robert Stier, Hollister-Stier Inc. Among the plants thus tested, several occur in Denmark while other frequent reactors are not indigenous (Table 2).

Materials for the standard patch tests were supplied by Trollab, A.N.Hansensvej, Hellerup. Denmark.

RESULTS

At Gentofte Hospital 6 of 10 apparently photosensitive patients, while at the Finsen Institute only 1 of 11 such patients, had positive reactions to Compositae oleoresins. The detailed results are shown in Tables 2 and 3.

TABLE 1. Age, sex and clinical history of the patients sensitive to Compositae oleoresins in petrolatum

Case no.	Age/sex Occupation	Duration and distribution of dermatitis	Previous diagnosis	Standard patch tests	Remarks
1	53/M Farmer	25 years Face, hands, neck behind ears (only summer)	Photodermatitis	Carba-mix 3% pet. ++ Thiuram-mix 1% pet. ++	Atopic asthma for many years, scratch test positive to house dust
2	72/M Railroad worker	50 years Face, behind ears, body, extremities (only summer)	Photodermatitis	Negative	
3	42/M ?	Flare up summer 1975 Face, neck, arms	Atopic dermatitis since childhood	Negative	Scratch tests positive to pollen of grasses
4	71/M Gardener	50 years Face, hands, arms (only summer)	Photodermatitis	Negative	
5	53/M Clerk	7 years Face, hands, neck behind ears (only summer)	Contact dermatitis photodermatitis	Nickel 5% pet. ++ Carba-mix 3% pet. ++ Thiuram-mix 1% pet. +++ Epoxy-resin 1% pet. +++ Colophony 20% pet. +++ Phenolformaldehyde-resin 5% pet. ++ Tricresylphosphate 5% pet. ++ p-tertiary butylphenol 50% in acetone ++ p-phenylenediamine 1% pet. ++ Balsam of Peru 25% pet. +	Hand eczema for 14 years with no seasonal variation
6	58/M Weaver	1 year Face, hands, arms (only summer)	Photodermatitis actinic reticuloid		
7	68/F Housewife	4 years Face, hands, arms, legs, back (only summer)	Contact dermatitis photodermatitis	Thiuram-mix 1% pet. ++	Hand eczema for 4 years with no seasonal variation

COMMENTS

Six of our seven Compositae sensitive patients had been treated for a period of 1–50 years with a diagnosis of photodermatitis (cf. Table 1). Compositae dermatitis of airborne pattern has, so far, been assumed to be an American phenomenon. As mentioned above it has also recently been reported from

TABLE 2. Test results in patients with positive reactions to Compositae oleoresins in petrolatum

Species	Case no.						
	1	2	3	4	5	6	7
**Chrysanthemum spp.* (chrysanthemum)	–	–	–	++	+++	+++	++
* *Centaurea cyanus* (cornflower)	–	–	–	–	+++	–	–
**Tanacetum vulgare* (tansy)	+++	+++	–	++	+++	+++	IR
**Artemisia spp.* (sagebrush)	IR	–	–	++	+++	–	NT
**Dahlia spp.* (dahlia)	–	–	–	++	++	–	–
**Solidago spp.* (goldenrod)	–	–	–	–	++	–	–
**Achillea millefolium* (yarrow)	+++	++	–	++	++	–	++
**Arctium minus* (burdock)	+++	–	–	NT	+++	–	–
**Cichorium intybus* (chicory)	+++	NT	–	–	+++	+	–
**Lactuca spp.* (lettuce)	++	NT	–	–	+++	–	NT
**Aster spp.* (aster)	–	NT	–	–	+++	–	–
Ambrosia trifida (giant ragweed)	++	–	–	–	–	+++	–
Parthenium hysterophorus (feverfew)	+++	–	–	++	++	+++	NT
Gaillardia pulchella (gaillardia)	+++	–	–	–	++	–	+++
Rudbeckia hirta (black-eyed Susan)	–	–	–	–	+++	–	–
Anthemis cotula (dog fennel)	+	–	–	–	++	–	–
Ambrosia artemisifolia (short ragweed)	+++	++	–	NT	–	++	+++
Coreopsis spp. (coreopsis)	++	–	+	++	+++	–	+
Xanthium strumarium (cocklebur)	+++	++	IR	++	+++	+++	IR
Erigeron strigosus (fleabane)	++	–	++	–	++	–	–
Iva ciliata (marshelder)	–	–	–	++	–	+++	–
Helenium autumnale (sneezeweed)	+++	–	–	++	+	+++	+++
Franseria spp. (false ragweed)	+++	++	++	++	+++	+++	++
Helianthus spp. (sunflower)	+++	NT	–	++	++	–	–
Photo patch tests	NT	neg	NT	neg	++†	NT	NT

* Common in Denmark; † *Ambrosia trifida*; IR, irritant reaction; NT not tested.

India and Australia and access to the experimental oleoresins series of Hollister-Stier has revealed that this type of dermatitis also occurs in Denmark, so it is probably a world-wide disease.

The airborne contact dermatitis from Compositae clinically resembles a lichenified photodermatitis (Figs 2 and 3). In photodermatitis a triangle behind the earlobe is characteristically spared (Wilkinson, 1976). In these cases it is involved, indicating that an airborne allergen causes the dermatitis.

Predisposing factors, e.g. active dermatitis, may be important for sensitization to occur. One, possibly two, of our Compositae sensitive patients had atopic dermatitis. A further patient had eczema and relevant positive patch tests to several allergens before the actual seasonal dermatitis.

It is an unsolved riddle why airborne Compositae dermatitis, like photo-allergic contact dermatitis from tribromosalicylanilide (Osmundsen, 1968) and actinic reticuloid (Ive *et al.*, 1969), should almost exclusively affect males.

Diagnosis. The diagnosis is based upon the clinical pattern of dermatitis and confirmed by closed patch testing with the Compositae oleoresins in petrolatum.

Our results contradict the findings of Calnan & Ramsay (1975). Nine male patients, aged 51–67 years, with a diagnosis of actinic reticuloid or photosensitive eczema, had negative *open* patch tests to Compositae oleoresins. In our experience open patch tests with Compositae oleoresins in acetone and alcohol give false negative reactions (Roed-Petersen & Hjorth, 1976).

TABLE 3. List of patients tested with *negative* results to Compositae oleoresins in petrolatum, but with dermatitis of light exposed areas

Age	Sex	Diagnosis	Remarks
		THE FINSEN INSTITUTE	
79	M	Actinic reticuloid	
68	M	Photo contact dermatitis Contact dermatitis	Photo patch tests positive to Irgasan DP 300, hexachlorophane and methdilazinum. Patch tests positive to p-aminobenzoic acid and sodium edetate
75	M	Atopic dermatitis	
52	M	Atopic dermatitis	
66	M	Pityriasis	
46	F	Atopic dermatitis	Atopic asthma
58	F	Atopic dermatitis	
79	F	Contact dermatitis	Patch tests positive to neomycin, nickel and formalin
52	F	Photodermatitis	
12	F	Atopic dermatitis	
		GENTOFTE HOSPITAL	
66	M	Contact dermatitis	
57	M	Photodermatitis	Free of symptoms after treatment with beta-carotene
45	M	Atopic dermatitis Contact dermatitis	Patch tests positive to balsam of Peru, carba-mix, cinnamic acid, mercaptobenzothiazole, hydrogenated woolfat and epoxy-resin. Scratch tests positive to pollen of grasses, birth and chrysanthemum
28	F	Urticaria	Hayfever. Scratch tests positive to pollen of grasses.

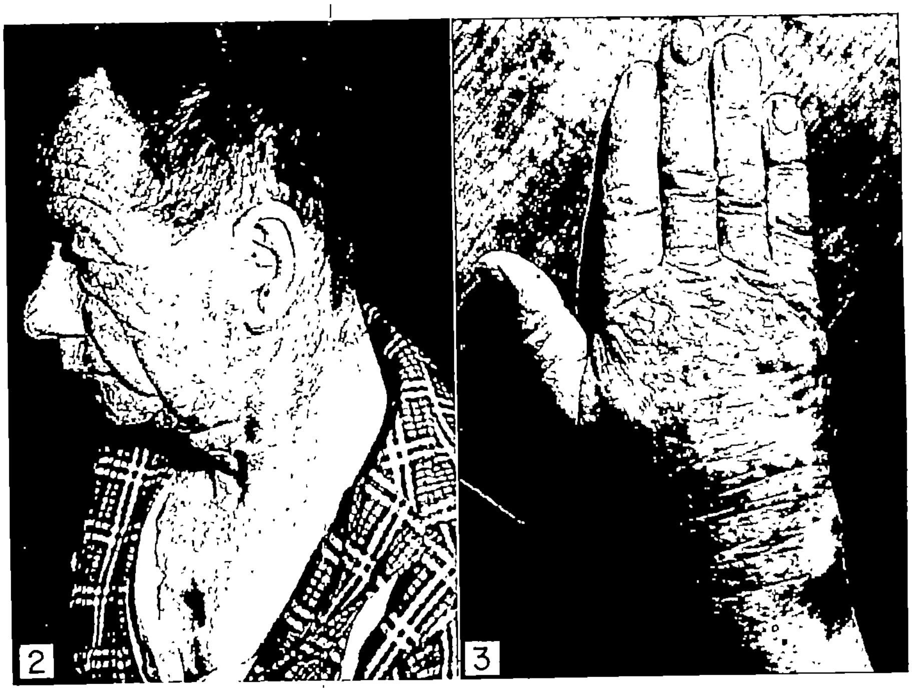
The allergenic species of the Compositae family contain sesquiterpene lactones with one common antigenic determinant (Fig. 1) which is a requirement for the sensitizing properties. However, not all compounds possessing this structure are immunologically active for all patients tested (Mitchell & Dupuis, 1971b), and the tulip allergen, which has a similar lactone ring, does not cross react (Mitchell, Dupuis & Geissman, 1972). Cross reactions between allergenic species in the Compositae family are common (cf. Table 2) since about 250 sesquiterpene compounds are distributed within this family (Mitchell *et al.*, 1972).

Patch testing would be simplified if a mixture of pure allergenic sesquiterpene lactones or an extract from one or more species could be used for screening. For the moment the latter method is not reliable. Pure alantolactone from *Inula helenium*, 1% in petrolatum, caused late reactions in 9 of 178 patients tested, and two patients known to be sensitive to Chrysanthemum had negative reactions (Hjorth, 1970a). In another series of 402 patients alantolactone, 0·1% in petrolatum, gave a positive reaction on day 2 and no flare-up reactions were observed (Hjorth, 1970b). In order to avoid active sensitization the patch test concentration of alantolactone therefore must not exceed 0·1%.

The liverwort *Frullania* contains sesquiterpene lactones related to alantolactone (Mitchell *et al.*, 1970). Storrs, Mitchell & Rasmussen (1976) found 2 of 12 *Frullania* positive patients negative to alantolactone.

We found only 3 of 7 patients sensitive to Compositae oleoresins to show a positive patch test reaction to alantolactone (Roed-Petersen & Hjorth 1976). Thus in a safe concentration alantolactone is not applicable for screening purposes.

Every patient seems to have his own pattern of positive reactions to Compositae oleoresins (Storrs *et al.*, 1976). This pattern is independent of exposure to the plants as appears from Table 2, which



FIGURES 2 and 3. Danish farmer with seasonal (summer) lichenified dermatitis of exposed areas. Sensitive to Compositae oleoresins in petrolatum.

shows as many positive reactions to common Danish Compositae species as to species not found or only found sporadically in Denmark. At present we are unable to state which are the commonest Compositae sensitizers in Denmark. An answer to this must await large-scale testing with extracts of common Danish species.

Further work with standardization of the test substances is required before the Compositae oleoresins can be recommended for general patch testing. All of our patients had positive reactions to Cocklebur (*Xanthium*), but controlled patch tests showed that this extract causes irritant reactions (Roed-Petersen & Hjorth, 1976). Another extract, Short Ragweed (*Ambrosia artemisifolia*), seemed perfectly safe when it was included in our standard patch test series in the summer of 1974, but when standard testing with the identical extract was resumed, many patients were patch test sensitized (Roed-Petersen & Hjorth, 1976).

The frequency of positive reactions warrants inclusion of Compositae in our standard series as soon as a safe reliable test material can be found. This is especially important because we have found many relevant but unexpected positive reactions to occur in patients with types of dermatitis other than the pattern here described (Roed-Petersen & Hjorth, 1976).

Treatment. At length—after years of treatment under a wrong diagnosis for an almost disabling dermatitis—the patients presented have a new and correct diagnosis after relevant patch testing. Are they happier for that reason? For the moment probably not, because we cannot offer these patients an effective treatment. It is impossible to avoid an ubiquitous allergen flying around in the air. Obviously, exposure is reduced if the patient moves from the country or suburb to the city. But this implies a change of social environment and possibly of occupation.

Kligman (1958) succeded in oral hyposensitization with Poison ivy (*Rhus*) extracts, but emphasized the side effects of this treatment. Oral hyposensitization with Compositae oleoresins is theoretically possible. Mitchell (1971b) gave alantolactone by mouth in increasing dosages to three patients with allergic contact dermatitis from sesquiterpene lactones and positive patch tests to alantolactone. He stated that the early results of such hyposensitization as shown by quantitative patch testing appeared promising, although further investigations were needed. Storrs *et al.* (1976) treated four Compositae sensitive patients, and two of these believed that their dermatitis was 'somewhat better' while taking oleoresin-filled capsules.

If a single species in a plant family causes an epidemic of contact dermatitis, as is the case with *Parthenium hysterophorus* in Poona, India, it is possible to mobilize a 'Destroy Parthenium Campaign' as described by Lonkar (1975). Because this Compositae dermatitis only affects men over 40, he used for his campaign students and school children to root out *Parthenium*.

Topical treatment with steroid creams could not control the dermatitis in our patients. Seasonal systemic steroid treatment must be considered—the risk of longterm side-effects is low because of the intermittent periods off steroids.

ACKNOWLEDGMENTS

Our thanks are due to Dr Niels Rosman for referring two of the patients reported and to Dr D.S. Wilkinson and to Dr John C. Mitchell for reviewing the manuscript.

REFERENCES

BURRY, J.N., KUCHEL, R., REID, J.G. & KIRK, J. (1973) Australian bush dermatitis: Compositae dermatitis in South Australia. *The Medical Journal of Australia*, **1**, 110.

CALNAN, C.D. & RAMSAY, C.A. (1975) Plant allergen test in photodermatitis. *Contact Dermatitis*, **1**, 122.

EPSTEIN, S. (1960) Role of dermal sensitivity in Ragweed contact dermatitis. *Archives of Dermatology*, **82**, 48.

EPSTEIN, S. (1972) Sensitivity to common weeds. *Contact Dermatitis Newsletter*, **11**, 305.

HJORTH, N. (1970a) Helenin. *Contact Dermatitis Newsletter*, **8**, 180.

HJORTH, N. (1970b) Active sensitization with alantolactone. *Contact Dermatitis Newsletter*, **8**, 175.

HOWELL, J.B. (1971) Sensitivity to common weeds. *Contact Dermatitis Newsletter*, **10**, 230.

IVE, F.A., MAGNUS, I.A., WARIN, R.P. & JONES, E.W. (1969) 'Actinic reticuloid': A chronic dermatosis associated with severe photosensitivity and the histological resemblance to lymphoma. *British Journal of Dermatology*, **81**, 469.

KLIGMAN, A.M. (1958) Hyposensitization against Rhus dermatitis. *Archives of Dermatology*, **78**, 47.

LONKAR, A., MITCHELL, J.C. & CALNAN, C.D. (1974) Contact dermatitis from *Parthenium hysterophorus*. *Transactions of the St John's Hospital Dermatological Society*, **60**, 43.

LONKAR, A. (1975) 'Destroy Parthenium' campaign. *Contact Dermatitis*, **1**, 390.

MITCHELL, J.C. (1969) Allergic contact dermatitis from Compositae. *Transactions of the St John's Hospital Dermatological Society*, **55**, 174.

MITCHELL, J.C., FRITIG, B., SINGH, B. & TOWERS, G.H.N. (1970) Allergic contact dermatitis from *Frullania* and Compositae. *The Journal of Investigative Dermatology*, **54**, 233.

MITCHELL, J.C., GEISSMAN, T.A., DUPUIS, G. & TOWERS, G.H.N. (1971a) Allergic contact dermatitis caused by *Artemisia* and *Chrysanthemum* species. *The Journal of Investigative Dermatology*, **56**, 98.

MITCHELL, J.C. & DUPUIS G. (1971b) Allergic contact dermatitis from sesquiterpenoids of the Compositae family of plants. *British Journal of Dermatology*, **84**, 139.

MITCHELL, J.C., DUPUIS, G. & GEISSMAN, T.A. (1972) Allergic contact dermatitis from sesquiterpenoids of plants. *British Journal of Dermatology*, **87**, 235.

OSMUNDSEN, P.E. (1968) Contact photodermatitis due to tribromsalicylanilide. *British Journal of Dermatology*, **80**, 228.

RODRIGUEZ, E., DILLON, M.O., MABRY, T.J., MITCHELL, J.C. & TOWERS, G.H.N. (1976) Dermatologically active sesquiterpene lactones in trichomes of Parthenium hysterophorus. *Experientia*, in press.

British Journal of Dermatology (1976) 95, 621.

Changes in dry weight and projected area of human epidermal cells undergoing keratinization as determined by scanning interference microscopy

H.J.YARDLEY AND D.J.GOLDSTEIN

MRC Unit on the Experimental Pathology of Skin, The Medical School, Birmingham 15, and Department of Human Biology and Anatomy, University of Sheffield

Accepted for publication 7 April 1976

SUMMARY

Cells, obtained from human leg by successive treatments with trypsin, were air dried on microscope slides before mounting in glycerol. Dry weights and projected areas of individual cells were measured using a Vickers M 86 scanning microinterferometer. The dry weights of cells varied from 100 pg for basal cells to 700 pg for large squames. Corresponding projected areas varied from 100 to 1500 μm^2.

That a keratinocyte increases in size as it progresses from the basal layer to the stratum corneum is well known, but few quantitative data are available. Even less is known about the changes in dry weight that occur when a cell undergoes keratinization. Hunter, Pinkus & Steele (1956) stated that the cells of the stratum corneum comprise about 10% of the total epidermal population, and according to Kligman (1964) the horny layer accounts for more than half of the dry weight of the epidermis. Putting these two facts together, we can derive a mean dry weight of cornified cells as more than nine times the dry weight of basal and spinous cells.

Meyer, Alvares & Barrington (1970) determined the average dry weight of cells in different layers of rat cheek and palate epithelium from separate measurements of cell density and cell volume. Density was measured by micro-dissecting frozen-dried epithelium, measuring the linear dimensions of the fragments and drying to constant weight. Cell volumes were estimated from photomicrographs of fixed, embedded and sectioned material. In cheek, the mean dry weight of basal cells was 156 pg, rising to 4700 pg (sic) in the granular layer and falling to 660 pg in the stratum corneum. In palate the corresponding figures were 100 pg for basal cells, 730 pg for granular cells and 404 pg for stratum corneum cells.

More recently, we have used a count-and-weigh method to derive a mean dry weight for cells obtained from pig epidermis by successive treatments with trypsin (Gray & Yardley, 1975b). The first crop of cells originated from the basal and spinous layers and had a mean dry weight of 276 pg. A second trypsinization usually yielded a mixed population of cells, but a third treatment consistently yielded a clean preparation of large cells containing about 10% of fully cornified cells. These large cells had a mean dry weight of 770 pg.

It has long been possible to measure, by microinterferometry, the dry mass per unit projected area of an individual cell (for reviews see Hale, 1958; Barer, 1966). Only recently, however, have instruments become commercially available which permit the rapid and precise measurement of the total dry mass of a heterogeneous microscopic object (Smith, 1967, 1972; Goldstein and Hartmann-Goldstein, 1974). In the present paper we describe the use of a scanning and integrating microinterferometer for determining the dry weights of individual cells derived from human epidermis.

METHODS

Preparation of epidermal cells

Skin was obtained from an amputated leg. Epidermal cells were obtained essentially by the method of Briggaman *et al.* (1967). Slices were taken from the skin with a Castroviejo keratotome (0·2 mm shim) (Blank, Rosenberg & Sarkany, 1961) and were floated, dermis side down, in a solution of trypsin (5 mg/100 ml; Sigma Chemical Co., type III) in phosphate-buffered saline (PBS; 137 mM NaCl, 2·68 mM KCl, 8·10 mM NaH_2PO_4, 1·47 mM KH_2PO_4) at pH 7·4 for 1·5–2 h at 37°C. The epidermis was peeled from the dermis, shaken in PBS containing trypsin inhibitor (5 mg/100 ml; Sigma type II O) to disperse the cells and filtered through nylon bolting cloth with a hole size of 0·02 × 0·02 mm (Nybolt 25 TII, Schweiz. Seidengazefabrik A.G., Zurich, Switzerland). The filtrate was centrifuged at 2000 rev/min (about 800 *g*) for 10 min, and the supernate discarded. The cells were resuspended in PBS and centrifuged again at 2000 rev/min for 10 min. This washing procedure was repeated until the cells were free from debris as judged by phase contrast microscopy. The cells were counted in a haemocytometer chamber and the average dry weight of a single cell was calculated from the weight of a freeze-dried sample of a known number of cells.

The residue on the nylon filter was thoroughly washed with PBS and again incubated in a solution of trypsin (5 mg/100 ml) in PBS at pH 7·4 for about 1·5 h at 37°C. The trypsin was inactivated by adding an equal volume of trypsin inhibitor (5 mg/100 ml in PBS), after which the mixture was shaken to disperse the cells and then filtered through nylon bolting cloth. This second crop of cells consisted of a mixture of basal, spinous and large nucleated cells and was washed as described earlier. A third crop of cells, consisting of very large nucleated cells and some fully cornified cells, was obtained by incubating with trypsin for a third time. Smears of the cell preparations were allowed to air dry on microscope slides before mounting in water or glycerol.

Measurement of dry weight and projected area

Isolated cells mounted in either water or glycerol were measured with a Vickers M86 scanning and integrating microinterferometer. This instrument automatically measures the optical path difference (OPD) between beams of light passing respectively through a small area of the specimen and a reference area containing only mounting medium. Measurements in glycerol were necessary for some cells (especially squames) because the OPD measuring range of the instrument, just under one wavelength of light, was sometimes insufficient when cells were mounted in water. Apart from pilot experiments, all measurements were therefore performed on cells mounted in glycerol. By scanning the object in a regular pattern (raster) the Vickers M86 records the integrated optical path difference (IOPD), which is proportional to the dry mass of the specimen (see below). In most of the work a × 40 objective was used, and each cell was measured at least twice, together with a similar number of blank readings of an empty area of background. Instrumental calibration (Goldstein & Hartmann-Goldstein, 1974) enabled measurements of IOPD in arbitrary machine units to be converted into absolute units having the dimensions of μm^3.

Measurements of IOPD in μm^3 units are converted into dry mass in pg (10^{-12} g) by dividing by

100α, where α is the mean specific refractive increments of the cell constituents (for review, see Barer, 1966). When using mercury green light (wavelength 546 nm), α is usually taken to be about 0·0018 for cells mounted in water. The value of α for cells mounted in glycerol is more controversial, published figures varying from 0·000645 to 0·00095, the most recent estimate being between 0·00084 and 0·00089 (Vöhringer & Maurer, 1971). Experimental data are unfortunately not yet available for values of α when using light of wavelength 632 nm (the helium–neon laser light used in the Vickers M86), but for present purposes we have taken the value of α to be the mean of the extreme values obtained by Vöhringer & Maurer (1971). Estimates of absolute dry mass thus obtained may subsequently require slight adjustment, but no substantial errors are expected from this cause, and measurements obtained in a given medium are of course directly comparable with each other.

The OPD of parts of some cornified cells was insufficiently different from the background to enable accurate measurements of projected cell area to be obtained using the automatic area-measuring facility of the M86. Projected areas of cells were therefore measured by planimetry of photographs.

RESULTS

Fig. 1 shows cell dry weight plotted against projected area. With small cells, projected surface area increases only slowly with cell mass. As the cells get larger and heavier, however, projected surface area increases more rapidly than mass. Fig. 1 also shows that for a given projected area, a cell with a nucleus or nuclear remnant tends to weigh more than a non-nucleated and therefore presumably more distal and older cell. Fig. 2 illustrates a range of cell sizes with corresponding dry weight. Occasionally, granule-containing cells were seen that had too high an OPD to measure. The average dry weight of a cell from the first trypsinization was 190 pg as determined by the count-and-weigh method. This value is consistent with our interferometric results. Insufficient cells were obtained from the second and third trypsinizations for determination by the count-and-weigh method.

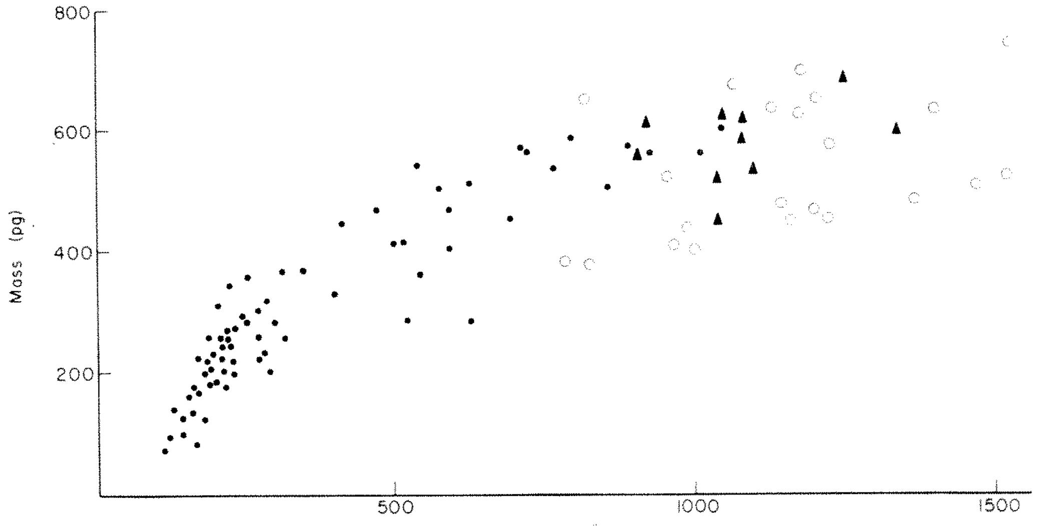


FIGURE 1. Mass and projected area of human epidermal cells. ● nucleated cells; ▲ cells containing a nuclear residue; ○ non-nucleated cells.

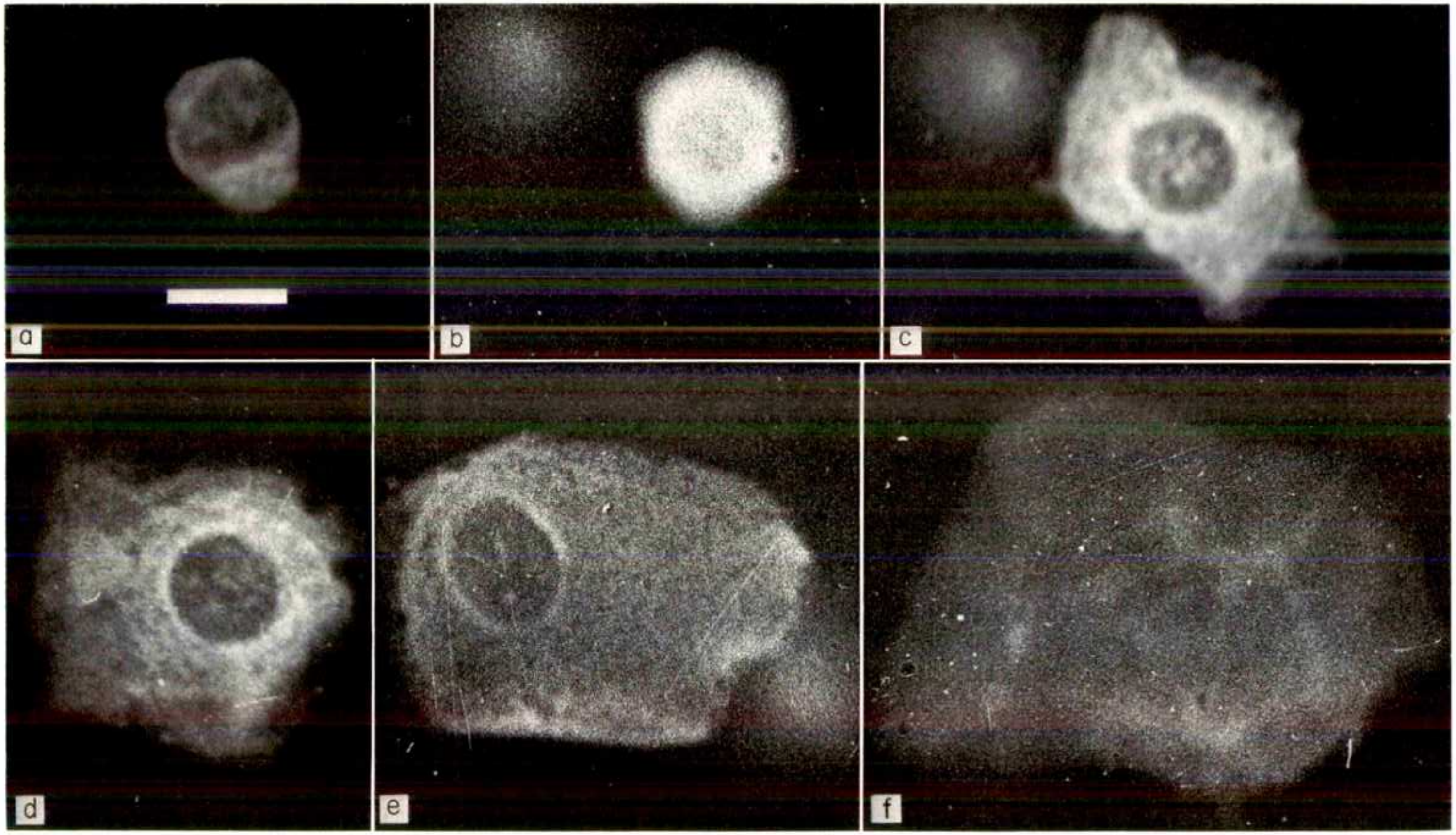


FIGURE 2. Photomicrographs of human epidermal cells taken with a Vickers M86 scanning micro-interferometer. Magnification is the same throughout; bar = 10 μm.

(a) projected area = 140 μm^2, dry weight = 130 pg.; (b) 170 μm^2, 180 pg; (c) 400 μm^2, 330 pg; (d) 690 μm^2, 460 pg.; (e) 790 μm^2, 590 pg.; (f) 1380 μm^2, 420 pg.

DISCUSSION

The projected area of an epidermal cell continuously increases as it moves from the basal layer to the stratum corneum. This has been demonstrated in neonatal rat (Stern & Sekeri-Pataryas, 1972), in pig tail (Gray & Yardley, 1975a) and in human leg (Gray & Yardley, 1975b). The present work shows that this increase in projected area is accompanied by an increase in dry weight. However, the dry weight is not proportional to the projected area, but varies in a way consistent with the view that keratinocytes maintain an approximately spherical shape while their mass increases from about 100 pg to about 400 pg, after which the cells continue to increase in weight but become progressively flatter. Having reached a maximum weight of 600–700 pg, the cells then lose mass to become fully cornified squames weighing 400–500 pg.

Meyer *et al.* (1970) arrived at qualitatively similar conclusions for epithelial cells of rat cheek and palate using an entirely different but somewhat questionable method. They measured density on freeze-dried tissue, but estimated volume on fixed and embedded material: they ignored the differences in the amount of shrinkage that occur when cells containing different concentrations of water are dehydrated during embedding. The value of 4700 pg obtained by Meyer *et al.* (1970) for the mean dry weight of granular cells seems extremely high, but their data for basal and stratum corneum cells are comparable with our results.

Our finding that the keratinocyte increases its mass several fold during the process of keratinization means that considerable synthesis must occur as the cell moves up through the epidermis, and there is published evidence (Bernstein, 1964; Stern & Sekeri-Pataryas, 1972) to indicate that protein synthesis occurs not only in the basal layer but also in the upper spinous and granular layers.

One application of our results would be to express chemical analyses of the various layers of the epidermis in terms of the amount of substance present per cell rather than the amount present per unit dry weight. For example, the lipid content of epidermal layers expressed as a percentage of the dry weight is essentially constant throughout the epidermis (Gray & Yardley, 1975b). This must mean that the amount of lipid per cell increases several fold as the cell moves from the basal layer to the stratum corneum and implies considerable lipid synthesis during the process of keratinization.

REFERENCES

BARER, R. (1966) Phase contrast and interference microscopy in cytology. In: *Physical Techniques in Biological Research* (Ed. by A.W.Pollister), 2nd edn, Vol. 3A, pp. 1–56. Academic Press, London.

BERNSTEIN, I.A. (1964) Relation of the nucleic acids to protein synthesis in the mammalian epidermis. In: *The Epidermis* (Ed. by W.Montagna and W.C.Lobitz), p. 471. Academic Press, London.

BLANK, H., ROSENBERG, E.W. & SARKANY, I. (1961) An improved technic for obtaining uniformly thin sheets of skin. *Journal of Investigative Dermatology*, **36**, 303.

BRIGGAMAN, R.A., ABELE, D.C., HARRIS, S.R. & WHEELER, C.E. (1967) Preparation and characterization of a viable suspension of post-embryonic human epidermal cells. *Journal of Investigative Dermatology*, **48**, 159.

GOLDSTEIN, D.J. & HARTMANN-GOLDSTEIN, I.J. (1974) Accuracy and precision of a scanning and integrating microinterferometer. *Journal of Microscopy*, **102**, 143.

GRAY, G.M. & YARDLEY, H.J. (1975a) Lipid compositions of cells isolated from pig, human and rat epidermis. *Journal of Lipid Research*, **16**, 434.

GRAY, G.M. & YARDLEY, H.J. (1975b) Different populations of pig epidermal cells; isolation and lipid composition. *Journal of Lipid Research*, **16**, 441.

HALE, A.J. (1958) *The Interference Microscope in Biological Research.* Livingstone, Edinburgh.

HUNTER, R., PINKUS, H. & STEELE, C.H. (1956) Examination of the epidermis by the strip method. *Journal of Investigative Dermatology*, **27**, 31.

KLIGMAN, A.M. (1964) The biology of the stratum corneum. In: *The Epidermis* (Ed. by W.Montagna and W.C. Lobitz), p. 387. Academic Press, London.

MEYER, J., ALVARES, O.F. & BARRINGTON, E.P. (1970) Volume and dry weight of cells in the epithelium of rat cheek and palate. *Growth*, **34**, 57.

SMITH, F.H. (1967) A photo-electronic phase-measuring microscope. *Journal of the Royal Microscopical Society*, **87**, 165.

SMITH, F.H. (1972) A laser illuminated scanning microinterferometer for determining the dry mass of living cells. *Microscope*, **20**, 153.

STERN, I.B. & SEKERI-PATARYAS, K.H. (1972) The uptake of ^{14}C-leucine and ^{14}C-histidine by cell suspensions of isolated strata of neonatal rat epidermis. *Journal of Investigative Dermatology*, **59**, 251.

VÖHRINGER, A. & MAURER, W. (1971) Messung der spezifischen optischen Streifenverschiebung bei interferometrischen Massenbestimmungen von fixiertem Eiweiss und Nukleoprotein. *Histochemie*, **25**, 234.

British Journal of Dermatology (1976) **95**, 627.

A lysosomal storage disorder of the epidermis characterized by a deficiency of α-mannosidase and an accumulation of mannose-rich materials

J.W.H.MALI, A.M.G.BERGERS, JOSÉ J.M.A.VAN DEN HURK,
P.D.MIER AND W.J.B.M.VAN DE STAAK

Department of Dermatology, University of Nijmegen, The Netherlands

Accepted for publication 10 May 1976

SUMMARY

Laboratory investigation of a patient diagnosed as ichthyosiform erythroderma bullosa revealed the following abnormalities:

(1) α- and β-galactosidases were elevated in the lesion. (2) α-mannosidase activity was extremely low both in the lesion and in relatively normal epidermis. (3) The mannose:glucose ratio of water-soluble hexose-containing material in the scales was very high. (4) Membrane-limited vacuoles, apparently enlarged secondary lysosomes, were observed.

We conclude that this patient is the first documented example of a lysosomal storage disease in which overt clinical lesions are confined to the epidermis.

The levels of 14 acid hydrolases in the lesions of some common dermatoses have recently been reported (Mier & van den Hurk, 1976). In general the most significant changes were non-specific elevations of the arylsulphatases and β-glucosidase, the magnitude of the increases being related to the degree of proliferation of the lesion. However, two patients diagnosed as ichthyosiform erythroderma (IE) were exceptional in showing raised levels of galactosidases and cathepsins. Because 'compensating' increases in certain of the unaffected hydrolases are a characteristic feature of the lysosomal storage diseases, our observation raised the interesting possibility that IE may be a member of this group, the hypothetical defect occurring in an enzyme which was not included in our range of assays.

We have now had the opportunity to investigate a single patient with a related condition, ichthyosiform erythroderma bullosa (IEB). The lesion of this patient also showed increased levels of α- and β-galactosidases, but, in contrast to the two cases of IE, a striking reduction in α-mannosidase activity was found. Our investigations on this patient are described below.

MATERIALS AND METHODS

The patient (W.H.S., male, aged 29 years) has suffered from a hyperkeratotic disorder with frequent bullous episodes since early childhood. He has attended this Department on a number of occasions since 1974; clinically and histologically his condition is consistent with the diagnosis of IEB. Except for his skin disease, the patient has always maintained normal health. His parents are unrelated and have no history of skin disease.

Epidermal slices were cut, stored and assayed for acid hydrolase activity as described previously (Mier & van den Hurk 1975a–c). Samples of scales were obtained from W.H.S. and from two psoriatic patients attending this Department; callus was scraped from the soles of two healthy volunteers. Scales and callus were defatted with ether, homogenized at 50 mg/ml in water, and centrifuged. The supernatant was brought to 1 N with HCl, heated in a sealed tube at 100°C for 3 h and neutralized. The hydrolysate was assayed for glucose, fructose and mannose using the NADP-linked system described by Gawehn (1974) but employing continuous fluorimetric measurement of NADPH. This modification provided a sensitivity of better than 0·2 nmol (2×10^{-10}mol) hexose per 0·5 ml sample of hydrolysate.

The specimens for electron microscopy were fixed, immediately after removal, in 2% glutaraldehyde and post-fixed in 2% osmium tetroxide. They were then embedded in Epon 812, ultra-thin sections were cut on a Reichert OM U2 ultramicrotome and stained with a saturated solution of uranyl acetate prior to being studied in a Philips EM 300.

RESULTS

Epidermal acid hydrolases. The levels of both α-galactosidase (21 nmol/min/g) and β-galactosidase (110 nmol/min/g) were significantly raised in a biopsy taken from an area of gross hyperkeratosis, but were normal in a biopsy from a region showing minimal clinical change. Both biopsies, however, were markedly deficient in α-mannosidase compared with healthy controls or with patients with other dermatoses (Table 1).

Water-soluble hexose-containing material in scales. The levels of glucose and mannose in hydrolysates

TABLE 1. Levels of α-mannosidase activity (nmol/min/g fresh weight) in epidermis. Values for controls and other diseases are taken from Mier & van den Hurk (1976)

Specimen	No. of samples	α-mannosidase activity
Healthy controls	46	28 (range 11–64)
All other diseases	55	30 (range 10–81)
W.H.S. (lesion)	1	5·9
W.H.S. ('uninvolved')	1	6·2

TABLE 2. Glucose and mannose in acid hydrolysates of aqueous extracts of scales and callus

Specimen	Glucose (μmol/g)	Mannose (μmol/g)	Mol mannose: 100 mol glucose
Callus			
control 1	1·2	0·056	5
control 2	1·9	0·015	1
Psoriatic scales			
patient 1	8·1	0·21	3
patient 2	1·9	0·27	14
W.H.S. scales			
sample 1	0·38	0·33	87
sample 2	2·4	1·9	79

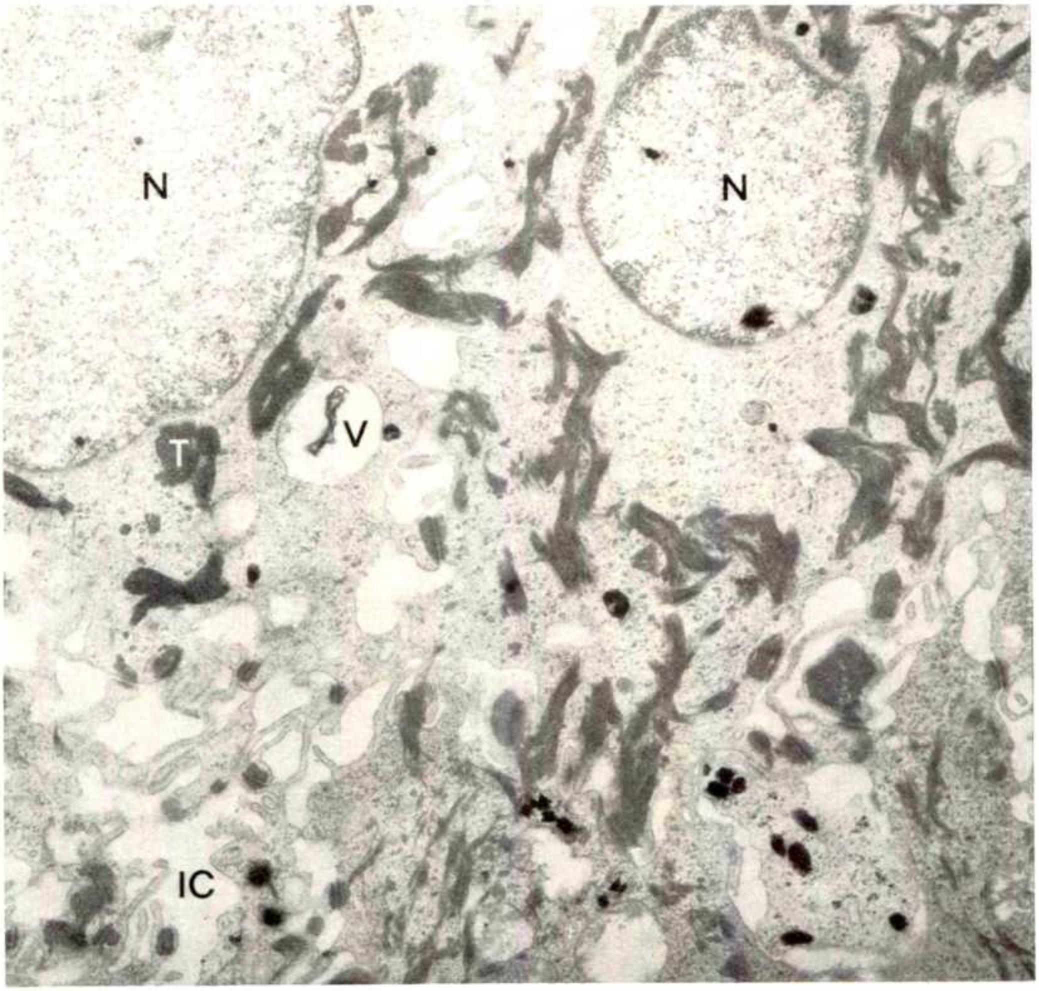


FIGURE 1. Epidermis from patient W.H.S. showing nuclei (N), tonofibrils (T), intercellular spaces (IC) and a typical membrane-limited vacuole (V) containing an inclusion body (× 16,200).

of water-extracts of scales and of callus are shown in Table 2. No fructose was found in any sample.

The wide range for absolute values (columns 1 and 2) presumably reflects variable losses resulting from washing or sweating prior to the collection of the specimens. Concentrations of mannose have therefore been expressed as mol mannose per 100 mol glucose (Column 3); this ratio was found to remain reasonably constant in duplicate samples. In these terms the mannose level of the scales from W.H.S. is seen to be more than five times that of any other sample examined.

Electron microscopy. Membrane-limited vacuoles were seen in many cells, being particularly numerous in the upper-regions of the spinous layer. Many of these contained membranous inclusion bodies (Fig. 1). The size of these vacuoles (averaging 1 μm) is typical of values reported for various storage conditions; Lloyd (1973), for example, comments that the maximum diameter of such storage vacuoles is remarkably constant, usually lying between 1 and 2 μm.

DISCUSSION

The enzyme α-mannosidase is one of a group of acid hydrolases concerned with the breakdown of glycoprotein. Although the precise sequence of events is still somewhat uncertain (and may not be identical for all glycoproteins), three stages are necessary. The initial step is the cleavage of the molecule by endopeptidases; the second is the removal of further amino acids by exopeptidases, and the third (probably occurring concurrently with the second) is the sequential removal of hexoses from the terminal reducing end of the oligosaccharides by specific glycosidases. Thus a defective glycosidase, such as α-mannosidase, will result in the accumulation of oligosaccharide fragments; these are water-soluble materials which liberate characteristic hexoses upon acid hydrolysis.

At least four criteria may be used to characterize a lysosomal storage disease. These include: (1) a reduced or absent acid hydrolase; (2) 'compensating' increases in related enzymes; (3) the accumulation of the appropriate metabolite; (4) the presence of enlarged secondary lysosomes. Since all four criteria have been fulfilled for a single patient with the clinical diagnosis of IEB, we may with some confidence claim the first demonstration of an epidermal disorder as a member of this group.

It is of particular interest to compare our findings with those reported in the brain, liver and spleen of patients with the systemic storage disease termed 'mannosidosis' (reviewed by Öckerman, 1973). Similarities include the reduction of α-mannosidase activity to about 20% of its normal level, an accumulation of water-soluble oligosaccharides yielding mannose on acid hydrolysis, and the demonstration of enlarged secondary lysosomes containing inclusion bodies resembling that illustrated in Fig. 1 (Autio *et al.*, 1973).

The relationship of our patient to the systemic disease may be understood in terms of the isoenzyme distribution of α-mannosidase (Carroll *et al.*, 1972). At least three forms of this enzyme exist in mammalian tissues: the A and B forms (pH optimum 4·4) and the C form (pH optimum 6·0). The A and B forms predominate in liver (and presumably other internal organs), whereas the more neutral pH optimum of the epidermal enzyme (Mier & van den Hurk, 1975a) indicates the latter to be mainly the C form. Consequently in systemic mannosidosis, in which the A and B forms are defective, the epidermis is spared. It is possible that in our patient precisely the converse situation obtains, the basic abnormality being a defect of mannosidase C. Further investigations regarding this point are in progress.

The inference that the clinically defined entity termed IEB is causatively related to an α-mannosidase abnormality must remain speculative until further patients are studied. Nevertheless, our present findings strengthen the possibility that the two patients with IE described previously (Mier & van den Hurk, 1976) also belong to the group of lysosomal storage diseases; obvious candidates for the defective hydrolase in this disorder are the remaining enzymes concerned with glycoprotein degradation, namely neuraminidase, α-fucosidase and N-aspartyl-β-glucosaminidase.

REFERENCES

AUTIO, S., NORDÉN, N.E., ÖCKERMAN, P.-A., RIEKKINEN, P., RAPOLA, J. & LOUHIMO, T. (1973) Mannosidosis: clinical, fine-structural and biochemical findings in three cases. *Acta paediatrica Scandinavica*, **62**, 555.

CARROLL, M., DANCE, N., MASSON, P.K., ROBINSON, D. & WINCHESTER, B.G. (1972) Human mannosidosis—the enzymic defect. *Biochemical and Biophysical Research Communications*, **49**, 579.

GAWEHN, K. (1974) D-mannose and D-mannose-6-phosphate. In: *Methods of Enzymatic Analysis* (Ed. by H.V.Bergmeyer), 2nd English edn, Vol. 3, p. 1263. Academic Press, New York and London.

LLOYD, J.B. (1973) Experimental support for the concept of lysosomal storage disease. In: *Lysosomes and Storage Diseases* (Ed. by H.G.Hers and F.van Hoof), p. 173. Academic Press, New York and London.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975a) Lysosomal hydrolases of the epidermis. 1. Glycosidases. *British Journal of Dermatology*, **93**, 1. *Idem* (1975b) 2. Ester hydrolases, **93**, 391. *Idem* (1975c) 3. Peptide hydrolases **93**, 509.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1976) Lysosomal hydrolases of the epidermis. 6. Changes in disease. *British Journal of Dermatology*, **95**, 271.

ÖCKERMAN, P.A. (1973) Mannosidosis. In: *Lysosomes and Storage Diseases* (Ed. by H.G.Hers and F.van Hoof), p. 292. Academic Press, NY and London.

British Journal of Dermatology (1976) **95**, 631.

Histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease

S.HAIM, J.D.SOBEL, R.FRIEDMAN-BIRNBAUM AND C.LICHTIG

Department of Dermatology and the Institute of Pathology, Rambam University Hospital and Aba Khoushi School of Medicine, Haifa, Israel

Accepted for publication 2 April 1976

SUMMARY

The mechanism of skin hyperreactivity (pathergy) in Behçet's disease is unknown. It has been suggested that the response is due to an Arthus-like reaction. In 18 patients with active Behçet's disease, pathergy was induced and the skin biopsied after 24 h. The histological reaction was that of mild to intense round cell infiltration, perivascular in type. Direct immunofluorescence failed in all 18 patients to demonstrate the local presence of immunoglobulins and complement at the site of lesion. A marked increase in the number of mast cells was observed at the site of reaction and scattered throughout the dermis, the role of which is to be further investigated. The study failed to demonstrate a humoral mechanism in the production of the cutaneous hyperreactivity.

The phenomenon of skin hyperreactivity in Behçet's disease is unique and is as yet inexplicable. Twelve to 48 h following intracutaneous injection of normal saline or a needle prick, an erythematous induration develops with sterile pus at its centre (Blobner, 1937; Jenson, 1941; Katzenellenbogen, 1968). The reaction is considered to reflect hypersensitivity provoked by minimal trauma and resulting in lesions similar to those occurring in the skin spontaneously in the disease. The time sequence of its development is somewhat late for an early type of reaction and early for a delayed one.

In an attempt to evaluate the role of humoral factors in the pathogenesis of this reaction and, possibly, of the disease itself, a histological and direct immunofluorescence study of this phenomenon was performed in 18 patients with active Behçet's disease.

SUBJECTS AND METHODS

There were 15 males and 3 females, ranging in age from 21 to 61 years, and the duration of their disease varied from 3 to 34 years. Eleven patients were born in Israel, 9 Arabs, 1 Druzian and 1 Jew. The remaining 7 were Jews, 3 of North African origin, 1 Egyptian, 1 Tunisian and 1 born in Poland. The diagnosis of Behçet's disease in these patients was based upon the presence of at least two of the three cardinal and one of the other manifestations recognized as part of process. Eight of the 18 presented the complete type of the disease. The remaining 10 lacked the ocular symptoms.

The skin reactivity in each patient was produced by intracutaneous injection into the forearm of 0·2 ml normal saline and by a needle prick performed simultaneously at a nearby site. Twenty-four hours later the site of the reaction was biopsied for histological and immunofluorescence examination. In addition to the above, in 4 patients normal skin and naturally occurring lesions were biopsied and similarly studied.

The preparations for immunofluorescence study were placed on a cryoform coated cork, and snap frozen in isopentane which had been precooled in dry ice. They were cut on the same day or stored at −20°C for no longer than 24 h. For the study we used antisera (Meloy Laboratories) against human IgA, IgG, IgM, IgE, C_3, C_4, albumin and fibrinogen. Purity of the antisera was verified by the production of a monospecific line on immunoelectrophoresis and by the Ouchterlony (1962) double diffusion method in agar. Blocking procedures with unconjugated antisera were included in the study. In few cases blocking studies were also done by incubating fluorescent antiserum with a purified mixture of IgA, IgG and IgM in which the antigen was in slight excess. In both techniques, the blocking resulted in either disappearance or marked reduction in the amount of specific fluorescence. All the preparations were examined with a Leitz Orthomat microscope with a dark-field condensor. A mercury bulb (Osram H.B.O. 200) was used as the source of light. The excitation filter was Leitz K.P. 490 interference filter and the barrier filter Leitz K. 490.

In all patients, the serum levels of C_3 and immunoglobulins were determined, as well as protein electrophoresis.

RESULTS

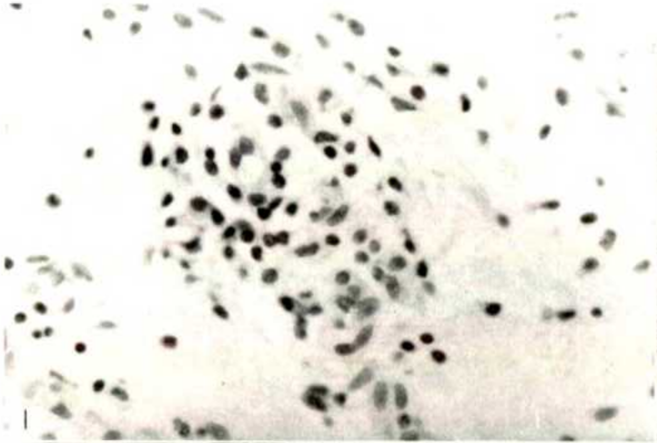
All patients responded positively and almost equally to both needle prick and the saline injection Within 24 hours an erythematous zone, 1–2 cm in diameter, developed with a pustule at its centre.

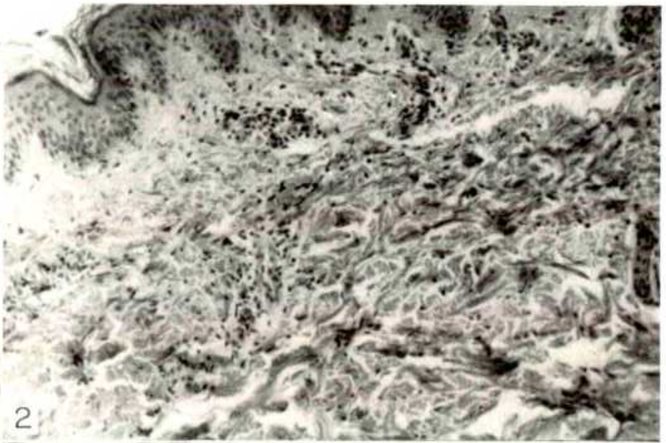
Histologically, the lesions revealed a variable amount of lymphocyte and plasma cell infiltration around the blood vessels in the upper dermis and around the skin adnexae (Fig. 1). The amount of cell infiltration varied from slight to intense. In some specimens, small clusters of neutrophils were also observed. No evident changes in the walls of the blood vessels were detected. Staining with toluidine blue, PAS and with NCA 'Naphthol—ASD—Chloroacetate Esterase' (Leder, 1964), demonstrated a marked increase in the number of mast cells. They were present in clusters at the site of inflammation but were also scattered throughout the dermis (Figs 2 and 3). These cells were normal in appearance; however also present in the cellular infiltrate were large mononuclear cells without granules in their cytoplasm which could represent degranulated mast cells. The histological findings of naturally occurring lesions in the mucous membrane and the skin were identical to those described in the skin hyperreactivity response (Fig. 4). Study of apparently normal skin during the active phase of the disease revealed either normal or slightly elevated numbers of mast cells with no other pathology.

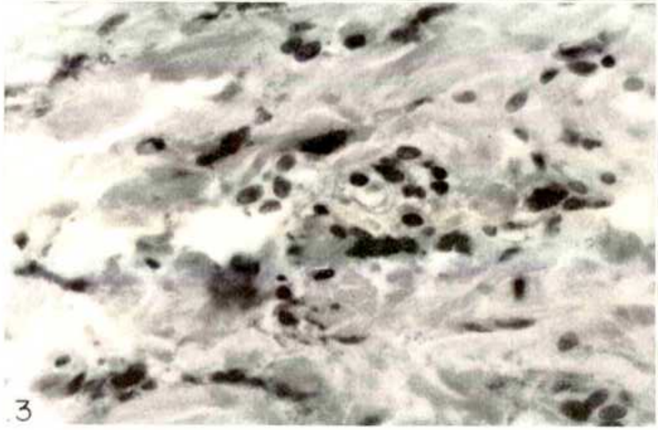
The direct immunofluorescence study showed no deposition of immunoglobulins IgA, IgG, IgM or IgE. There was also no deposition of C_3, C_4, fibrinogen or albumin.

Immunoglobulin estimations showed minimal increase in IgG, IgA and IgM as well as a slight increase in alpha$_2$ globulin. The range of serum IgG level was 15–20 mg/ml, IgA 2–5 mg/ml, IgM 2–3 mg/ml and of alpha$_2$ globulin 1·27–1·81 g/ml. The normal accepted ranges in our laboratory are 6·6–14·6, 1·8–4·2, 0·4–1·8 mg/ml and 0·4–1·0 g/ml respectively.

Serum C_3 level was normal, although in 7 patients the values obtained were around the lower limit of normal.







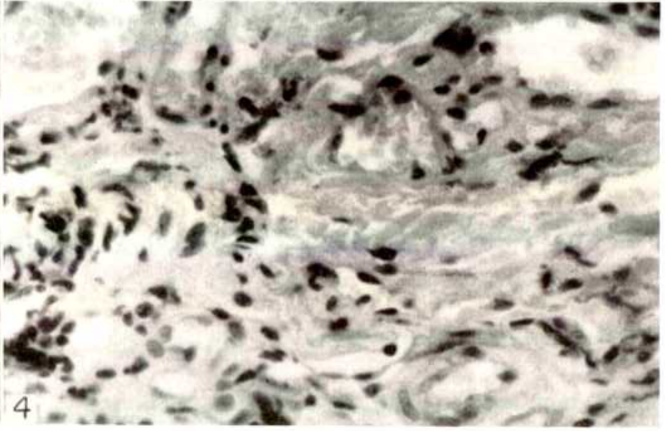


FIGURE 1. Small vessel in the upper dermis surrounded by mononuclear cell infiltrate. No signs of vasculitis are seen (H & E, × 500).

FIGURE 2. Reactive cutaneous lesion revealing round cell infiltrate and increased number of mast cells (NCA, × 125).

FIGURE 3. Many granulated and degranulated mast cells at the site of the reaction (NCA, × 500).

FIGURE 4. Skin lesion revealing large number of small mononuclear cells, many of them lymphocytes (NCA, × 500).

DISCUSSION

Nazzaro (1966), in a study of skin, mucous membrane and scrotal lesions produced by needle prick in cases with Behçet's disease, described vascular changes in addition to perivascular infiltration. He reported thickening and homogenization of the wall of the small blood vessels, at times with hyaline thrombosis of the lumen. Others described the vascular changes as mild or absent (France *et al.*, 1951; Ship, Merritt & Stanley, 1962). The histological findings of the reaction to needle prick or to intracutaneous saline injection in our cases, revealed a perivascular predominantly round cell infiltrate 24 h later. No significant granulocytic infiltrate was noted and apart from the perivascular distribution of the cell infiltrate there were no signs of vasculitis *per se*. The granulocytic clusters revealed in few specimens were probably a direct result of tissue damage due to injection.

The skin reactions were biopsied 24 h after induction, somewhat arbitrarily, in the presence of developed lesions. At present, a study is in progress to investigate the microscopic picture 12, 24, 48 and 72 h after trauma in order to obtain a more complete and dynamic understanding of the histological response.

In the absence of an adequate explanation for skin hyperreactivity some authors have suggested that this response represents, histologically, the expression of an Arthus-like reaction in the production of cutaneous vasculitis (Cooper, Penny & Fiddes, 1971). In a further attempt to verify this hypothesis, direct immunofluorescence was used to identify any immunoglobulin, fibrin or complement at the site of the reaction.

Direct immunofluorescence has been extremely useful in the detection of antibodies in the skin in a variety of diseases in which the presence of local antibody correlated well with the activity of skin disease in the absence of detectable serum antibodies (Van Joost, 1975).

The evidence in favour of an immune pathogenesis in Behçet's disease is unconvincing. Although Oshima *et al.* (1963) and subsequently Lehner (1967a) and O'Duffy, Corney & Deodhar, (1971) found circulating antibodies to human oral mucosa, Lehner (1964) found the same frequency of these antibodies in patients suffering from aphthous stomatitis. The latter antibodies may well be the result of non-specific destruction of mucosa, especially since no correlation was found between antibody titre and activity of the disease.

In none of the 18 patients with active Behçet's disease studied was any evidence found of the local presence of immunoglobulins or complement, suggesting that local humoral factors are not relevant in the pathogenesis of cutaneous hyperreactivity lesions or perhaps of the mucocutaneous lesions.

Studies of the humoral system in Behçet's disease have not as yet identified a clear pathogenetic mechanism. During relapse a transient increase in serum levels of alpha$_2$ globulin as well as IgA and IgG occur (Chajek & Fainaru, 1975; Lehner, 1972). Likewise, elevated levels of alpha$_2$ globulin, IgG, IgA as well as IgM were found in this series. This suggests stimulation of B lymphocytes during the acute inflammatory stage of the disease but does not prove a causal effect. Elevated total haemolytic complement activity has been reported in cases with Behçet's disease (Kogure, Shimada & Hara, 1971), and Kawachi-Takahashi *et al.* (1974) found a marked increase in C_9 levels in 21 of 27 patients. The C_3 level, as confirmed in our cases, has been reported to be normal (Chajek & Fainaru, 1975; Lehner, 1972).

The literature reveals evidence incriminating a cell mediated mechanism in the pathogenesis of Behçet's disease (Lehner, 1972). This is based mainly on the following features demonstrated in the process: induction of lymphocyte transformation by oral mucosa (Lehner, 1967b); cytotoxic effect of lymphocytes to oral mucosa (Dolby 1969; Rogers, Sams & Shorter, 1974); delayed hypersensitivity type of skin reaction to skin homogenates (Jadassohn *et al.*, 1961; Katzenellenbogen & Feuerman, 1965); and the histological features characterized by an early intense lymphocytic infiltration (Coe *et al.*, 1966; Graykowski *et al.*, 1966).

However, it should be pointed out that lymphocytic transformation and lymphocyte induced cytotoxicity in the presence of tissue antigens is not definitive proof of a causal relationship. This may be the result of lymphocyte sensitization following tissue injury. Furthermore, the induction of cutaneous reaction in Behçet's disease with skin homogenates may similarly be caused by non-antigenic factors such as trauma. Nevertheless, Lehner (1969a) reported correlation between cell-mediated immunity and the activity of the disease. In this context we like to mention a study of lymphocyte function in Behçet's disease recently conducted in our department. The results of the study were suggestive of a possible impairment of the regulatory mechanism which controls T cell proliferation in this process (Haim *et al.*, 1976).

Lehner (1969b) described a three-fold rise in the number of mast cells in the cellular infiltrate of recurrent oral ulcers, including Behçet's, in contrast to their decreased count in non-specific ulcers of the mouth. Our study, likewise, revealed a significantly increased number of mast cells in the area of the reactive inflammatory response as well as in the infiltrate of naturally occurring lesions in the skin and mucous membrane. It is worthy of note that these cells were present in normal or slightly elevated numbers in the normal skin from patients in an active stage of the disease.

Normally, the dermis contains only a small number of mast cells, encountered mainly in the vicinity of capillaries and skin appendages (Lever, 1967; Heilwing & Mostofi, 1971). Their number in the dermis is increased in a variety of conditions apart from mastocytosis such as granulation tissue of healing wounds, keloids, a number of chronic dermatoses, tumours both benign and malignant, etc. (Wichmann, 1955; Asboe-Hansen, 1964). However, their presence in large numbers in new reactive lesions suggests a possible role for these cells in the production of this cutaneous response and requires further investigation.

REFERENCES

ASBOE-HANSEN, G. (1964) Connective tissue response to injury. In: *Injury, Inflammations and Immunity* (Ed. by L. Thomas, J.W. Uhr and L. Grant), p. 22. Williams and Wilkins, Baltimore.

BLOBNER, F. (1937) Zur rezidivieren Hypopion-Iritis. *Zeitschrift Augenheilk*, **91**, 129.

CHAJEK, T. & FAINARU, M. (1975) Behçet's disease, report of 41 cases and a review of the literature. *Medicine*, **54**, 180.

COE, J.E., FELDMAN, J.D. & LEE, S. (1966) Immunologic competence of thoracic duct cells. I. Delayed hypersensitivity. *Journal of Experimental Medicine*, **123**, 267.

COOPER, D., PENNY, R. & FIDDES, P. (1971) Autologous-plasma sensitisation in Behçet's disease. *Lancet*, **i**, 910.

DOLBY, A.E. (1967) Recurrent aphthous ulceration: effect of sera and peripheral blood lymphocytes upon oral epithelial tissue culture cells. *Immunology*, **17**, 709.

FRANCE, R., BUCHANAN, R.M., WILSON, M.W. & SHELDON, M.B. (1951) Relapsing iritis with recurrent ulcers of mouth and genitalia (Behçet's syndrome): review with report of additional case. *Medicine*, **30**, 335.

GRAYKOWSKI, E.A., BARILE, M.F., LEE, W.E. & STANLEY, H.R. (1966) Recurrent aphthous stomatitis. *Journal of the American Medical Association*, **196**, 637.

HAIM, S., MEKORI, T., SOBEL, J.D. & ROBINSON, E. (1976) Aspects of lymphocyte function in Behçet's disease. *Dermatologica*, in press.

HEILWING, E.B. & MOSTOFI, F.K. (1971) *The Skin*, p. 95. Williams and Wilkins, Baltimore.

JADASSOHN, W., FRANCESCHETTI, A. & HUNZIKER, N. (1961) On the Behçetin reaction. *Hautarzt*, **12**, 64.

JENSON, T. (1941) Sur les ulcerations aphtheuses de la muquesus de la bouch et de la peu genitale combinees avec les symptomes oculaires (syndrome Behçet). *Acta dermato-venereologica*, **22**, 64.

KAWACHI-TAKAHASHI, S., TAKAHASHI, M., KOGURE, M. & KAWASHIMA, M. (1974) Elevation of C_9 level associated with Behçet disease. *Japanese Journal of Experimental Medicine*, **44**, 488.

KATZENELLENBOGEN, I. (1968) Survey of 22 cases of Behçet's disease: 'the significance of specific skin hyperreactivity'. *XIII Congressus Internationalis Dermatologiae* (*München*, 1967), p. 321. Springer, Berlin.

KATZENELLENBOGEN, I. & FEUERMAN, E.J. (1965) Die Bedeutung der spezifischen Hauthyperreactivitat und der Behçetin Reaction. *Hautartz*, **16**, 13.

KOGURE, M., SHIMADA, K. & HARA, H.G. (1971) Complement titer in patients with Behçet's disease. *Acta Societatis Ophthalmologica, Japan*, 75, 1260.

LEDER, L.D. (1964) The selective enzymocytochemical demonstration of neutrophil myeloid cells and tissue mast cells in paraffin sections. *Klinische Wochenschrift*, **42**, 553.

LEHNER, T. (1964) Immunological aspects of recurrent oral ulcers. *Lancet*, **ii**, 80.

LEHNER, T. (1967a) Behçet's syndrome and autoimmunity. *British Medical Journal*, **i**, 465.

LEHNER, T. (1967b) Stimulation of lymphocyte transformation by tissue homogenate in recurrent oral ulceration. *Immunology*, **13**, 159.

LEHNER, T. (1969a) Characterisation of mucosal antibodies in recurrent aphthous ulceration and Behçet's syndrome. *Archives of Oral Biology*, **14**, 843.

LEHNER, T. (1969b) Pathology of recurrent oral ulceration and oral ulceration in Behçet's syndrome. Light electron and fluorescence microscopy. *Journal of Pathology*, **97**, 481.

LEHNER, T. (1972) Immunological aspects of recurrent oral ulcers. *Oral Surgery*, **33**, 80.

LEVER, W.F. (1967) *Histopathology of the Skin*, 4th edn, p. 53. J.B. Lippincott, Philadelphia.

NAZZARO, P. (1966) Cutaneous manifestations of Behçet's disease: clinical and histological findings. *Proceedings of the International Symposium on Behçet's Disease*, p. 15. Karger, Basel.

O'DUFFY, J. D., CORNEY, J.A. & DEODHAR, S. (1971) Behçet's disease: report of 10 cases, 3 with new manifestations. *Annals of Internal Medicine*, **75**, 561.

OSHIMA, Y., SHIMIZU, T., YOKOHARI, R., MATSUMATO, T., KANO, K., KAGAMI, T., NAGAYA, H. & MARUGAMA, R. (1963) Clinical study on Behçet's syndrome. *Annals of Rheumatic Diseases*, **22**, 36.

OUCHTERLONY, O. (1962) Diffusion in gel methods for immunological analyses. *Progress in Allergy*, **6**, 30.

ROGERS, R.S., SAMS, W.M. & SHORTER, R.G. (1974) Lymphocytotoxicity in recurrent aphthous stomatitis: Lymphocytotoxicity for oral epithelial cells in recurrent aphthous stomatitis and Behçet's syndrome. *Archives of Dermatology*, **109**, 361.

SHIP, I.I., MERRITT, A.D. & STANLEY, H.R. (1962) Recurrent aphthous ulcers. *American Journal of Medicine* **32**, 32.

VAN JOOST, TH. (1975) Circulating autoantibodies in skin disease: a survey and comparison of immunofluorescence (IF) studies. *International Journal of Dermatology*, **14**, 379.

WICHMANN, P.E. (1955) The mast cell count during the process of wound healing. *Acta Pathologica et Microbiologica Scandinavica*, Suppl. **180**, 1.

British Journal of Dermatology (1976) **95**, 637.

Familial Behçet's syndrome

S.K.GOOLAMALI,* J.S.COMAISH, F.HASSANYEH†
AND A.STEPHENS†

University Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP
and † St George's Hospital, Morpeth, Northumberland

Accepted for publication 28 May 1976

SUMMARY

Behçet's syndrome is reported in a family of which four generations have so far been affected. The index patient also exhibited an unusual schizo-affective disorder. The histocompatibility antigen haplotype 1–17 was common to four with the disease. Genetic transmission may be responsible for the familial nature of the syndrome and the associated schizophrenia.

The aetiology of Behçet's syndrome is unknown. Behçet (1937) himself suggested a viral cause for the disease now named after him, on the basis of finding inclusion bodies in scrapings from mouth ulcers. Isolation of a virus has been claimed (Sezer, 1953; Evans, Pallis & Spillane, 1959) and an autoimmune aetiology has also been postulated because of the presence of antibodies against oral mucosa (Lehner, 1967; Oshima *et al.*, 1963) and the cytoplasm of polymorphonuclear leukocytes (Shimizu, Katsuda & Oshima, 1965).

To our knowledge genetic transmission has not been implicated in the pathogensis of the disease. We now report the syndrome in a family of which four generations have so far been affected. Two brothers suffered in addition from psychiatric disorders, while their mother had severe alopecia areata also apparently genetically transmitted. The pedigree of the family is illustrated in Fig. 1 and shows that five members were affected by Behçet's syndrome in a family group of eleven.

CASE REPORTS

Patient 1. E.W.

This 26-year-old man was first seen in the Skin Clinic in January 1973, with a history of recurrent mouth ulceration since the age of 2 years. Later this was associated with genital ulceration and inflammatory lesions of the eyes. As a child he also suffered from recurrent upper respiratory tract infections, and on one admission for elective tonsillectomy at the age of 3 years was noted to have generalized lymphadenopathy, hepatomegaly and splenomegaly. These abnormalities persisted for at least 5 months. No cause for this was found and a month later tonsillectomy was completed without incident. Childhood neurotic traits were extensive and prominent, and he received speech therapy for a stammer. At the age of 26, as an unemployed single man with an established schizoid personality, he developed progressive loss of drive and confidence, becoming increasingly withdrawn and anxious. In September

---

* Present appointment: Consultant Dermatologist, Central Middlesex and Wembley Hospitals, London and Northwick Park Hospital, Middlesex.

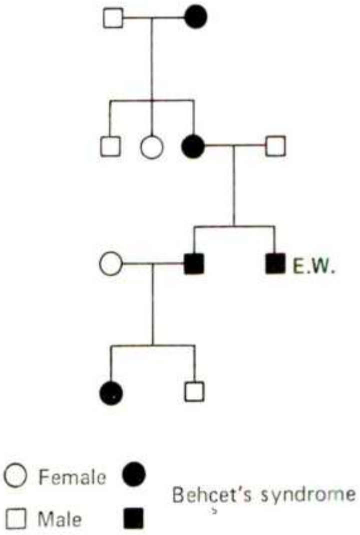


FIGURE 1.

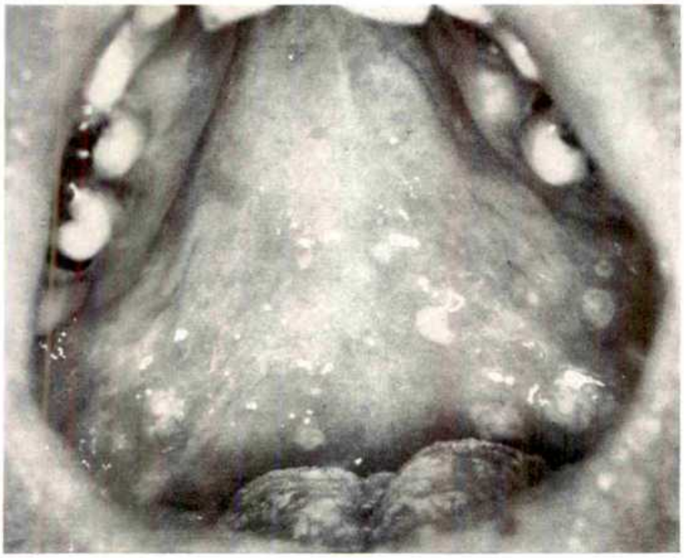


FIGURE 2.

FIGURE 1. Family tree showing involvement of 4 generations.
FIGURE 2. Multiple oral ulcers with scarring (case E.W.).

1973 he was admitted compulsorily for psychiatric care with gross psychotic features. These included early morning waking; ideas of reference, failure, worthlessness and self-injury; complete auditory hallucinations, both direct and discussing him critically in the third person; thought-disorder; thought-broadcasting; and bizarre thought content. Clinically, his mental state varied unpredictably with depression, lachrymosity, retardation, and perplexity. Preoccupied silences, grimacing, and resistiveness were observed.

A diagnosis of acute schizophrenia with affective features was established, and recovery took place slowly over a period of 5 months after treatment with ECT, phenothiazines and antidepressants.

General examination showed bilateral conjunctivitis and several small shallow necrotic ulcers of the aphthous type in the mouth with scarring of the mucosa (Fig. 2). He also had scrotal ulceration and as an in-patient was noted to develop necrotic lesions at the sites of venepunctures over the dorsa of the hands (Fig. 3). The rest of the clinical examination was negative and in particular there was no lymphadenopathy or hepatosplenomegaly.

Investigations

Histology of scrotal ulcer. There was chronic inflammation of the dermis with prominent blood vessels. Some sections showed an acanthotic epidermis, and necrosis with some ulceration.

Other investigations. Laboratory investigations produced no evidence of haematological, biochemical, or serological abnormality apart from an ESR of 30 mm/h (Westergren) and a slight increase in α_2 globulin. WR, VDRL, and Reiter's Protein CFT were negative, as were viral studies and antinucleoprotein factor. Cryoglobulins were not detected and leukocyte myeloperoxidase, glucose 6-phosphate dehydrogenase, red cell catalase, euglobulin lysis time and fibrinogen levels were all normal. The pressure and constituents of the cerebrospinal fluid were normal. A standard EEG produced a low amplitude beta-dominant trace with no focal, lateralising or paroxysmal anomalies.

Comment

For the past 12 years this patient has received prednisone 5–10 mg daily. Although this dosage has

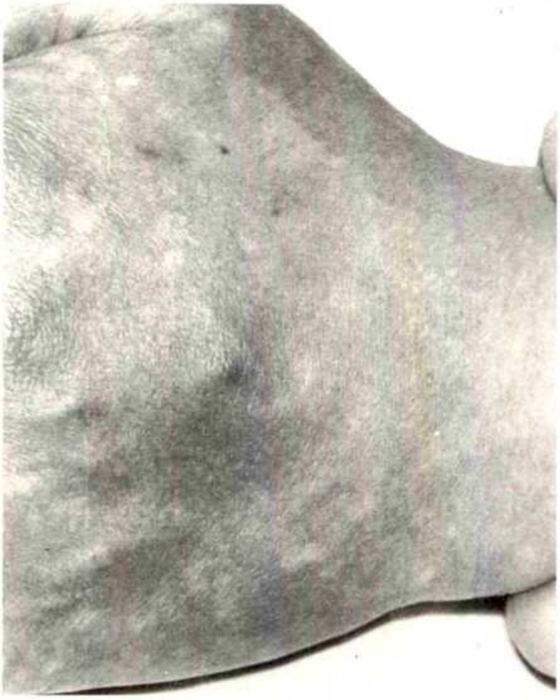


FIGURE 3. Inflammatory papules developing at site of venepuncture (case E.W.).

reduced the number of exacerbations of the Behçet's syndrome, he has nevertheless experienced acute episodes necessitating substantial increases, up to 60 mg daily, in his prednisone therapy. He has never suffered from superficial or deep vein thrombosis, arthritis, arthralgia or organic central nervous system complications.

Patient 2, M.W. aged 64 years, mother of E.W.

She was diagnosed as suffering from Behçet's syndrome in June 1966 when she presented with ulceration of the mucous membrane of the cheeks, tongue and a small ulcer on the vulva. She had a severe exacerbation in 1969 and in 1970 an episode of herpes simplex of the lower lip. Her progress otherwise has been uneventful with occasional mouth ulceration only. She describes the onset of Raynaud's phenomenon several years ago and has also received treatment for 'rheumatoid' arthritis in the past. In addition she developed severe alopecia areata 20 years ago and there has been no regrowth of hair.

Examination

She was a cushingoid lady with mouth ulcers and scarring of the fauces. There were no active eye or genital lesions. The rest of the clinical examination was also negative.

Investigations

ESR 80 mm in the first hour: serum proteins—total 1·8 g%, albumin 3·6 g%, globulin 4·5 g%. Abnormal Electrophoretic strip with an increase in α_2 and gammaglobulin fractions.

Urinalysis, Wassermann reactions, Coombs' test, cold agglutinins, antinuclear factor, cryoglobulins and platelet counts were normal.

Comment

This patient is maintained on betamethasone sodium phosphate (Betnesol) 0·25 mg daily. The raised ESR and hyperglobulinaemia are consistent with Behçet's syndrome.

R.W. (brother of E.W.)

He, aged 34 years, and one of his two children, a daughter aged 7 years, have also exhibited recurrent ocular and oral lesions.

M.S. (maternal grandmother of E.W.)

She is reported by her daughter to have suffered from recurrent severe mouth ulceration and inflammation of the eyes for some 50 years. In addition she suffered from polyarthropathy, Raynaud's syndrome and an untreated bereavement depression. At her death in 1946 in hospital she was noted to have peripheral neuropathy.

Blood groups and HLA antigens

Examination of the blood grouping and human leukocyte antigens (HLA) (Fig. 4) shows a remarkable similarity between E.W. and his brother as they differ only in the P blood-group. The histocompatibility antigens are also identical to those found in the niece of E.W. The HLA haplotype 1–17 is common to four suffering from Behçet's syndrome. The father also has HLA 17 though neither of his sons has inherited this from him.

E.W.	B	ccDEE	MNss	P_{1+}	kk	Fy^{a-b+}	Jk^{a+b+}	Lu^{a-b+}	Le^{a-}	HLA A1-BW17, A2-B12
Brother	B	ccDEE	MNss	P_{1-}	kk	Fy^{a-b+}	Jk^{a+b+}	Lu^{a-b+}	Le^{a-}	HLA A1-BW17, A2-B12
Father	B	ccDEe	MMSs	P_{1+}	kk	Fy^{a+b+}	Jk^{a+b+}	Lu^{a-b+}	Le^{a-}	HLA A2-BW17, A2-B12
Mother	O	ccDEE	NNss	P_{1+}	kk	Fy^{a-b+}	Jk^{a+b+}	Lu^{a+b+}	Le^{a-}	HLA A1-BW17, A9-B27
Niece of E.W.	O	CcDEe	NNss	P_{1+}	kk	Fy^{a-b+}	Jk^{a+b+}	Lu^{a-b+}	Le^{a-}	HLA A1-BW17, A2-B12
Nephew of E.W.	O	CcDEe	NNss	P_{1-}	kk	Fy^{a-b+}	Jk^{a-b+}	Lu^{a-b+}	Le^{a-}	HLA A2-B12

FIGURE 4. Blood groups and histocompatibility antigens.

Family psychiatric history

R.W., brother of the index patient, was handicapped by nervousness and shyness in childhood, and was sensitive to bullying and intimidation at school. At the age of 24, he received treatment for an anxiety state from his general practitioner, with subsequent referral to a psychiatric out-patient clinic. The dominant features included tension, phobias, lack of confidence, insomnia, headaches and dyspepsia. At the age of 31 he responded temporarily to hypnotherapy for similar complaints. He attached particular importance to stress as a precipitating or exacerbating factor in his personal history of recurrent oral ulceration.

Neither parent showed any significant psychiatric abnormality in a structured interview, and gave no history of previous psychiatric treatment. E.W.'s maternal grandmother (with Behçet's syndrome) was reported to have suffered from a prolonged untreated bereavement depression. A paternal granduncle received continuous psychiatric in-patient care for 34 years for chronic schizophrenia, corroborated by case records. The paternal great grandmother was of a nervous disposition and over anxious. She committed suicide at the age of 60.

DISCUSSION

The diagnosis of Behçet's syndrome may be made when at least two of the three major criteria of recurrent oral ulcers, genital ulcers and uveitis and/or other ocular manifestations are present in the patient's history, or physical examination, or both (Curth, 1946; Mounsey, 1966). Arthritic manifestations such as relapsing hydrarthrosis, arthralgia and arthritis are quite common in Behçet's syndrome as is involvement of the vascular system (Dowling, 1961; France *et al.*, 1951). With the exception of the lower motor neuron, any part of the central nervous system may also be involved.

Dementia has been described in patients with neurological signs, but there have been few reports on the psychiatric manifestations of Behçet's syndrome. Schotland *et al.* (1963) referred to nine patients in whom signs of confusion and emotional lability were present. Individual case reports have recorded a 'hypersensitive' or 'immature' personality as being associated with the syndrome (Chalmers & Sircus, 1964; Murphy, 1970). In a study designed to observe the psychiatric aspects of Behçet's syndrome (Epstein *et al.*, 1970) it was noted that patients exhibited submissiveness, depression and neuroses.

These trends are reflected in the personalities and psychiatric disorders observed in the family group described here. The development of acute schizophrenia with affective features in one of our patients does not appear to have been previously reported, however, nor does the unusual concurrence of psychiatric and dermatological abnormalities among first degree relatives, in the manner illustrated by the two brothers. In the index case, the diagnosis of acute schizophrenia rests on a florid psychosis with unequivocal Schneiderian first-rank and affective symptoms arising from an abnormal premorbid personality.

Published descriptions of familial incidence of Behçet's syndrome are rare. Sezer (1956) diagnosed the syndrome in three brothers, and neurological manifestations with Behçet's syndrome have also been described in two sisters (Fowler *et al.*, 1968). Mason & Barnes (1969) reported 4 patients in whom other members of the family were affected, and in these up to two generations had the disease. More recently a review of 45 patients with Behçet's syndrome in the United Arab Republic (Fadli & Youssef, 1973) showed that in three instances two siblings had the disorder. We believe our study is the first report of familial Behçet's syndrome extending to four generations.

The aetiology of Behçet's syndrome is unknown though the association between HLA phenotypes and certain diseases including Behçet's syndrome (Ohno *et al.*, 1973) may be significant. A close functional relationship has been demonstrated between histocompatibility specificities and the immune response, controlled by the immune response (Ir) gene, both in the guinea-pig (Shevach, Paul & Green, 1972) and the mouse (Shreffler, 1974). In the mouse this response is linked to the H-2 system which is the counterpart of the HLA system of man. The relationship of HLA phenotypes with human diseases suggests that similar Ir-type genes are linked to the HLA system. A very high incidence of the histocompatibility antigen W27 has been found in patients with Reiter's disease (Brewerton *et al.*, 1973a) and acute anterior uveitis (Brewerton *et al.*, 1973b). Despite the clinical similarity between Reiter's disease and Behçet's syndrome we did not find the W27 antigen in any of our patients. The histocompatibility haplotype 1–17 was found, however, to be common to four with the disease. The familial nature of the disease would explain this prevalence and this common antigen could in turn influence the response to an 'infection' or auto-antigens, both of which are possible aetiological agents in Behçet's syndrome.

A viral cause has generally been discounted, but recent reports on slow viruses and their possible aetiological roles in entities such as Kuru (Gajdusek, Gibbs & Alpers, 1967) and Jacob–Creutzfeld disease (Gibbs, Gajdusek & Asher, 1968) point to the possibility of a similar mechanism in Behçet's syndrome. The persistent and unexplained lymphadenopathy and hepatosplenomegaly in our patient E.W. during childhood may represent the occasion when viral invasion occurred. The familial nature of the syndrome in our patients, however, also suggests the possibility of genetic transmission. The close similarity between the histocompatibility antigens in three of the affected patients is also in keeping with a genetic aetiology. Genetic factors of unknown effect and transmission are generally held to be of aetiological importance in schizophrenia, and may be operating in the family described. The possibility of a chance association between Behçet's syndrome and psychiatric disorders in the brothers E.W. and R.W., mediated independently, is to some extent supported by their paternal family history of suicide and schizophrenia.

ACKNOWLEDGMENTS

We wish to thank Drs J. Cotterill and N. Rowell, Consultant Dermatologists, Leeds General Infirmary for helpful clinical information regarding E.W. and M.W. Dr D.F. Roberts, Department of Human Genetics, University of Newcastle kindly completed the blood groupings and histocompatibility tests on our patients.

REFERENCES

BEHÇET, H. (1937) Ueber rezidivierende, Aphthose, durch einen virus verursachte Geschwure am Mund am Auge und den Genitalien. *Dermatologische Wocheschrift*, **105**, 1152.

BREWERTON, D.A., CAFFREY, M., NICHOLS, A., WALTERS, D., OATES, J.K. & JAMES, D.C.O. (1973a) Reiter's disease and HL-A27. *Lancet*, **ii**, 996.

BREWERTON, D.A., CAFFREY, M., NICHOLS, A., WALTERS, D. & JAMES, D.C.O. (1973b) Acute anterior uveitis and HL-A27. *Lancet*, **ii**, 994.

CHALMERS, D. & SIRCUS, W. (1964) Hypnosis in the treatment of recurrent aphthous stomatitis. *Gut*, **5**, 599.

CURTH, H.O. (1946) Recurrent genito-oral aphthosis and uveitis with hypopyon (Behçet's syndrome). *Archives of Dermatology and Syphilology*, **50**, 179.

DOWLING, G.B. (1961) Behçet's disease. *Proceedings of the Royal Society of Medicine*, **54**, 101.

EPSTEIN, R.S., CUMMINGS, N.A., SHERWOOD, E.B. & BERGSMA, D.R. (1970) Psychiatric aspects of Behçet's syndrome. *Journal of Psychosomatic Research*, **14**, 161.

EVANS, H.D., PALLIS, G.A. & SPILLANE, J.D. (1959) Involvement of nervous system in Behçet's syndrome. Report of three cases and isolation of virus. *Lancet*, **ii**, 349.

FADLI, M.E. & YOUSSEF, M.M. (1973) Neuro-Behçet's syndrome in the United Arab Republic. *European Neurology*, **9**, 76.

FOWLER, T.J., HUMPSTON, D.J., NUSSEY, A.M. & SMALL, M. (1968) Behçet's syndrome with neurological manifestations in two sisters. *British Medical Journal*, **ii**, 473.

FRANCE, R., BUCHANAN, R.N., WILSON, M.W. & SHELDON, M.B. (1951) Relapsing iritis with recurrent ulcers of the mouth and genitalia (Behçet's syndrome). Review with report of additional case. *Medicine (Baltimore)*, **30**, 335.

GAJDUSEK, D.C., GIBBS, C.J. & ALPERS, M. (1967) Transmission and passage of experimental 'kuru' to chimpanzees. *Science*, **155**, 212.

GIBBS, C.J., GAJDUSEK, D.C., ASHER, D.M., ALPERS, M.P., BECK, E., DANIEL, P.M. & MATTHEWS, W.B. (1968) Creutzfeld–Jakob disease (spongiform encephalopathy) transmission to the chimpanzee. *Science*, **161**, 388.

LEHNER, T. (1967) Behçet's syndrome and autoimmunity. *British Medical Journal*, **i**, 465.

MASON, R.M. & BARNES, C.G. (1969) Behçet's syndrome with arthritis. *Annals of Rheumatic Diseases*, **28**, 95.

MOUNSEY, J.P.D. (1966) Behçet's syndrome: a spectrum. *Transactions of the St John's Hospital Dermatological Society*, **52**, 197.

MURPHY, D. quoted by Epstein *et al.* in Psychiatric aspects of Behçet's syndrome (1970). *Journal of Psychosomatic Research*, **14**, 161.

OHNO, S., AOKI, K., SUGIURA, S., NAKAYAMA, E., ITAKURA, K. & AIZAWA, M. (1973) HL-A5 in Behçet's disease. *Lancet*, **ii**, 1383.

OSHIMA, Y., SHIMIZU, T., YOKOHARI, R., MATSUMATO, T., KANO, K., KAGAMI, T., NAGAYA, H. & MARUGAMA, R. (1963) Clinical study on Behçet's syndrome. *Annals of Rheumatic Diseases*, **22**, 36.

SCHOTLAND, D.L., WOLF, S.M., WHITE, H.H. & DUBIN, H.V. (1963) Neurologic aspects of Behçet's disease. Case report and review of the literature. *American Journal of Medicine*, **34**, 544.

SEZER, N. (1953) Isolation of virus as a cause of Behçet's disease. *American Journal of Ophthalmology*, **36**, 301.

SEZER, N. (1956) Further investigations on the virus of Behçet's disease. *American Journal of Ophthalmology*, **41**, 41.

SHEVACH, E.M., PAUL, W.E. & GREEN, I. (1972) Histocompatibility-linked immune response gene function in guinea-pigs. Specific inhibition of antigen-induced lymphocyte proliferation by alloantisera. *Journal of Experimental Medicine*, **136**, 1207.

SHIMIZU, T., KATSUDA, Y. & OSHIMA, Y. (1965) Immunological studies on Behçet's syndrome. *Annals of Rheumatic diseases*, **24**, 494.

SHREFFLER, D.C. (1974) Genetic fine structure of the H-2 gene complex. In: *Cellular Selection and Regulation in the Immune Response* (Ed. by G.M.Edelman), p. 83. Raven Press, New York.

British Journal of Dermatology (1976) **95**, 643.

Pharmacology and Treatment

Relapse rate of psoriasis worsened by adding steroids to a dithranol regime*

R.H.SEVILLE

Department of Dermatology, Beaumont Hospital, Lancaster

Accepted for publication 29 March 1976

SUMMARY

The rate of relapse after dithranol therapy is significantly less than when betamethasone valerate under occlusion is combined with dithranol in the treatment of psoriasis. It is also argued that local steroids are inferior to dithranol in the management of psoriasis.

To be able to clear psoriasis and to keep it clear as long as possible must be the dermatologist's aim. Treatment should be judged not only on how long the skin stays clear but also on the ease and simplicity of use at home and its lack of immediate or delayed toxicity. Local therapy is usually by dithranol, tar, steroids or combinations of these applications. It has already been shown (Seville, 1966) that even a weak dithranol 0·125% paste with salicylic acid 2% was significantly quicker in clearing psoriasis than the traditional tar therapies; a simplified dithranol therapy with a stiff ointment that was easier to use at home has since been described (Seville, 1975).

Relatively rapid relapse after the use of potent local steroids was commented on by Champion (1966) and Juhlin (1967), and also by Stevenson & Whittingham (1963) and Alexander (1965) who noted that it was less when dithranol therapy was used in addition. A combination of steroid and dithranol might therefore have the advantages of speed of clearance and a relapse rate possibly comparable with dithranol therapy.

Psoriasis is a constitutional affection with a tendency to vary and relapse bilaterally and patient management is more effective with simple regimes. It was thought that a straightforward comparison of relapse rates in completely cleared matched patients would indicate differences between therapies more clearly. This paper briefly describes work undertaken in 1968 which ought to be re-emphasized as even more potent local steroid preparations are now available.

MATERIALS AND METHOD

Twenty-eight patients who were routine consecutive admissions were treated with dithranol and steroid. While most of the treatment had been undertaken previously with dithranol 0·5% paste it was soon found that when alternated with local steroids the strength had to be reduced, otherwise severe treatment reactions occurred. A weaker preparation was therefore used, namely dithranol 0·25% with salicylic acid 1% in emulsifying ointment B.P. and was alternated on a daily basis with betamethasone valerate 0·1% ointment under polythene occlusion for about 18 h at a time. Treatment was stopped only when the skin was completely clear. Either dithranol paste 0·1% or crude coal tar paste 3·4% was then applied daily for about 4 days in an attempt to prevent relapse. Ultraviolet light and tar baths were given in addition. Personal follow-up was undertaken and the time of relapse established by careful questioning. The word 'clear' was defined to include also those patients where full examination revealed the occasional minute lesion or patch of roughness in the scalp of which the patient was often unaware.

---

* Based on a presentation to the North of England Dermatology Society in Lancaster, June 1975.

The patients treated with steroid and dithranol were then matched for age, sex and number of previous admissions with patients cleared only with dithranol 0·5% in stiff Lassar's paste, ultraviolet light and tar baths. The last-mentioned patients, who had been carefully followed-up, were taken from a series where the treatment had been standardized and where it was ultimately shown that deletion of ultraviolet light, and tar from the bath, did not affect treatment and relapse time (Seville, 1975). It was only in this series that a personal assessment of the extent of the eruption on admission was available, so only extensive cases were chosen to give a bias against the dithranol therapy. Retrospective assessment from the notes of the 28 patients treated with steroid and dithranol show that only six patients would have been classified as extensive (i.e. more than 50% involvement).

RESULTS

The mean in-patient time for the steroid/dithranol group was 3·4 weeks including the 4 days time after clearance, as compared with the 4·0 weeks for the paired dithranol group. The mean relapse time was 5·3 weeks in the former and 27·9 weeks in the latter, but this excludes one patient found to be clear at 3-year follow-up; the difference is statistically significant ($P = 0{\cdot}01$) and is shown in Fig. 1.

DISCUSSION

In 1960 patients were originally asked to record which side was better in a double-blind comparison of dithranol paste. After nine consecutive patients had chosen the side that was doing badly, it transpired that they all preferred the side where there was the least stain. Because of this the opinion

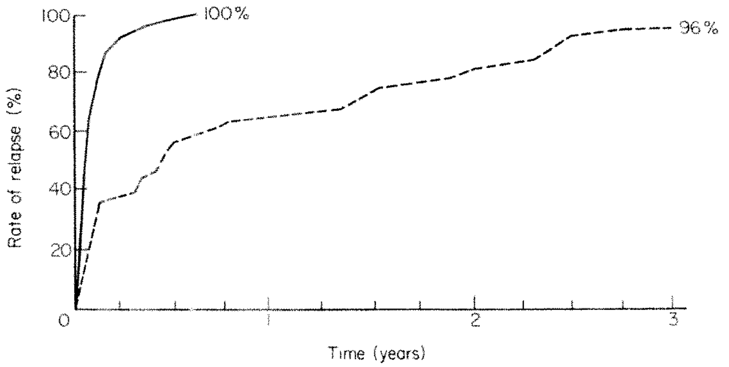


FIGURE 1. Rate of relapse of patients using dithranol/steroid (——), and using dithranol alone (- - - -). There are 28 patients in each group. The graph shows a more rapid rate of relapse with local steroids plus dithranol than in the extensive psoriatics treated with dithranol.

of the patients was not asked in subsequent trials but care was taken to explain to them the importance of stain as an indication of the effectiveness of the dithranol treatment. The rate of relapse however is of much greater importance than problems from staining and the difference can be seen from studying Fig. 1.

Despite the matched patients treated with dithranol being more extensively affected than those treated with local steroids and dithranol, the rate of relapse was strikingly slower with dithranol on its own. Therapy was carried out for a mean time of 0·6 weeks longer with the dithranol-treated patients. This difference is not considered important as Frank *et al.* (1964) had used steroid and polythene for 7 weeks to achieve a similar relapse rate at 6 months follow-up. Furthermore their patients had been selected by including only those who were clinically clear at 3 weeks and had put up with the routine at home for a further month, there being a greater number of defaulters than those who had completed the course.

The same tendency to relapse after local steroids was noted by Knudsen (1965) who was unable to prove it statistically in hemilateral experiments with fluocinolone under occlusion, compared with paintings with coal or diluted wood tars; comment was made that absorption of the steroid would also increase the chance of rebound on the non-steroid side, so making proof more difficult. Suurmond (1967) found no difference in relapse rate with triamcinolone occlusion and dithranol but again the comparison was hemilateral; also the dithranol was in zinc paste which would have been less effective than if salicylic acid had been present (Comaish, Smith & Seville, 1971).

It has been shown that transient pituitary adrenal suppression follows the use of fluorinated steroids especially under polythene occlusion (James, Munro & Feiwel, 1967; Munro, Feiwel & James, 1967; Feldman & Maibach, 1967). Staughton & August (1975) mentioned that clobetasol 0·05% may exert systemic effects far beyond those of other preparations; Carruthers, August & Staughton (1975) commented that because of systemic absorption from steroid creams such as clobetasol, physicians should observe as much caution with their use as with systemic corticosteroids. Repeating the present trial was unjustifiable with the newer steroids as they were considered similar in action to less potent ones used under polythene. The above-mentioned studies on the systemic effects of topical steroids, the cumulative local changes and particularly the development of patient dependence should severely restrict their use; this paper demonstrates their inferiority in the management of psoriasis compared with dithranol therapy.

ACKNOWLEDGMENTS

I am indebted to Drs P.J.August, D.B.Brookes, S.Comaish and G.B.Walker for helpful comments, to Mrs Shirley Barber for the calculations, Miss Dorothy Till and Mrs Hilary Newman for the typing and to Mr Grantham and his nursing staff for constant team work.

REFERENCES

ALEXANDER, S. (1965) The treatment of psoriasis with steroid creams under polythene. *British Journal of Dermatology*, **77**, 163.

CARRUTHERS, J.A., AUGUST, P.J. & STAUGHTON, R.C.D. (1975) Observations on the systemic effect of topical clobetasol propionate (Dermovate). *British Medical Journal*, **ii**, 203.

CHAMPION, R.H. (1966) Treatment of psoriasis. *British Medical Journal*, **ii**, 993.

COMAISH, S., SMITH, J. & SEVILLE, R.H. (1971) Factors affecting the clearance of psoriasis with dithranol (anthralin). *British Journal of Dermatology*, **84**, 282.

FELDMAN, R.V. & MAIBACH, H.I. (1967) The penetration of radio-labelled hydrocortisone in human skin *in vivo*. *Proceedings of the XIII Congressus Internationalis Dermatologiae*, p. 186.

FRANK, L., STEINER, K., BENDER, B. & WINSTON, M. (1964) Flurandrenolone and occlusion in psoriasis. *Archives of Dermatology*, **89**, 404.

JAMES, V.H.T., MUNRO, D.D. & FEIWEL, M. (1967) Pituitary–adrenal function after occlusive topical therapy with betamethasone-17-valerate. *Lancet*, **ii**, 1059.

JUHLIN, L. (1967) Effects of topical steroids in normal and psoriatic skin. *Proceedings of the XIII Congressus Internationalis Dermatologiae*, p. 198.

KNUDSEN, E.A. (1965) Fluocinolone-plastic treatment and traditional treatment in severe psoriasis. A comparative study. *Acta dermato-venereologica*, **45**, 50.

MUNRO, D.D., FEIWEL, M. & JAMES, V.H.T. (1967) The influence of topical corticosteroids on the hypothalamic–pituitary–adrenal axis. *Proceedings of the XIII Congressus Internationalis Dermatologiae*, p. 202.

SEVILLE, R.H. (1966) Dithranol paste for psoriasis. *British Journal of Dermatology*, **78**, 269.

SEVILLE, R.H. (1975) Simplified dithranol treatment for psoriasis. *British Journal of Dermatology*, **93**, 205.

STAUGHTON, R.C.D. & AUGUST, P.J. (1975) Cushing's syndrome and pituitary–adrenal suppression due to clobetasol propionate. *British Medical Journal*, **ii**, 419.

STEVENSON, C.J. & WHITTINGHAM, G.E. (1963) Psoriasis treated with topical fluocinolone acetonide and occlusive dressings. *British Medical Journal*, **i**, 1450.

SUURMOND, D. (1967) The recurrence rate of psoriatic lesions after topical treatment with dithranol and corticosteroids under plastic dressings. *Proceedings of the XIII Congressus Internationalis Dermatologie*, p. 200.

British Journal of Dermatology (1976) **95**, 647.

Case Reports

Epidermal naevus syndrome

M.K.ATTIA* AND A.M.ABDEL-AZIZ†

* Department of Dermatology, Maadi Armed Forces Hospital, and
† Department of Dermatology and Venereology, Al-Azhar University, Cairo, Egypt

Accepted for publication 22 March 1976

SUMMARY

A case of the epidermal naevus syndrome is presented. Huge enlargement of both lower limbs was the presenting symptom together with an extensive naevus unius lateris on the trunk.

The neurologist Schimmelpenning (1957) defined with some precision the already known association between epidermal naevi and epilepsy, mental retardation and ocular defects. The association was further publicised in the English-speaking world by Feuerstein & Mims (1962). Since then many cases have demonstrated the association of epidermal naevi with other developmental defects, constituting probably not one but several different syndromes.

In this paper we describe a case of the epidermal naevus syndrome in which there were very marked skeletal abnormalities.

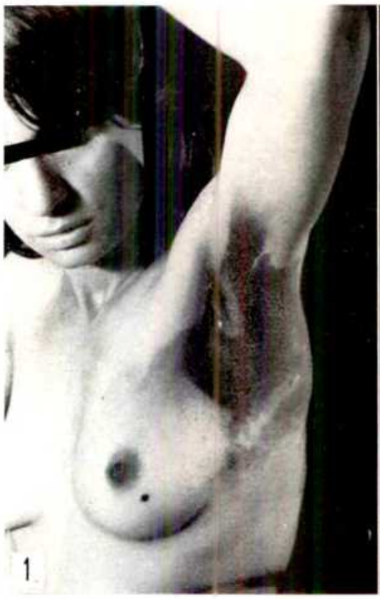
CASE REPORT

A 19-year-old female presented with huge enlargement of both lower limbs from birth. There was no similar condition in the family and no consanguinity. Her weight was 73 kg and height 168 cm. A large warty epidermal naevus involved the left axilla and adjacent areas of the chest anteriorly and posteriorly. No café-au-lait marks were present. Both lower limbs were enlarged, above and below the knee, the right more than the left (Table 1). Her feet were grossly enlarged, although the big toes were relatively normal in size. Apart from the huge enlargement of both lower limbs and the skin lesion, the other systems of the body showed no abnormality.

Investigations. X-rays of both feet showed large second, third and fourth metatarsals and phalanges of both feet. An arteriogram of both lower limbs showed no abnormality in size or number of vessels. E.M.G.s of the right extensor hallucis longus, tibialis anterior and both heads of the gastrocnemius muscle showed no spontaneous activity and contraction provoked a normal interference pattern. Blood count, ECG, EEG and X-rays of the chest and all other bones of the body were normal.

COMMENT

Solomon, Fretzin & Dewald (1968) in their report on the associated findings in 23 patients with the epidermal naevus syndrome found skeletal abnormalities in 18. Two of these patients had enlarged limbs and localized gigantism, but full details were not given.



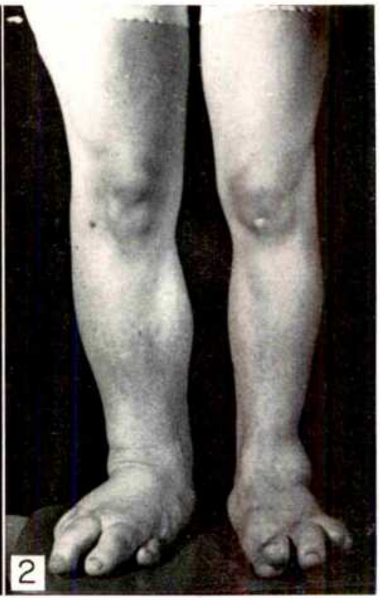


FIGURE 1. Left axilla showing epidermal naevus.

FIGURE 2. Massive enlargement of both lower limbs.

TABLE 1. Dimensions of limbs

Circumference of right thigh	62 cm
Circumference of left thigh	54 cm
Circumference of right calf	34 cm
Length of right leg (ant. sup. iliac spine to med. malleolus)	89 cm
Length of left leg (ant. sup. iliac spine to med. malleolus)	83 cm
Length of right foot (heel to big toe)	34 cm
Length of left foot (heel to big toe)	24 cm

The commonest association between skeletal overgrowth and skin changes is with neurofibromatosis and with the Klippel–Trenaunay–Weber syndrome. In our case there is nothing to suggest neurofibromatosis, and the angiogram of both limbs showed no abnormality in the size or number of the vessels.

Skeletal hypertrophy may sometimes be due to an increased blood supply resulting from a primary developmental abnormality of the circulatory system such as an arteriovenous fistula in the Klippel–Trenaunay–Weber syndrome. In our case the angiogram of both lower limbs showed no abnormality in size or number of the vessels.

REFERENCES

Feuerstein, R.C. & Mims, L.A. (1962) Linear nevus sebaceus with convulsions and mental retardation. *American Journal of Diseases in Childhood*, **104**, 675.

Schimmelpenning, G.W. (1957) Klinischer Beitrag zur Symptomatologie der Phakomatosen. *Fortschritte auf dem Gebiete der Röntgenstrahlen und der Nuklearmedizin*, **87**, 716.

Solomon, L.M., Fretzin, D.F. & Dewald, R.L. (1968) The Epidermal nevus syndrome. *Archives of Dermatology*, **97**, 273.

British Journal of Dermatology (1976) **95**, 649.

Atrophie blanche lesions closely resembling malignant atrophic papulosis (Degos' disease) in systemic lupus erythematosus

MARTIN M. BLACK AND PETER M. HUDSON

Department of Dermatology, St Thomas' Hospital, London SE1 7EH

Accepted for publication 26 April 1976

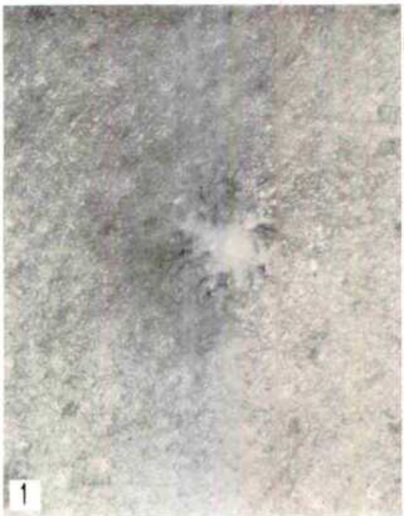
SUMMARY

Two patients with systemic lupus erythematosus are described who in the course of their disease developed small atrophie blanche lesions that closely resembled those found in malignant atrophic papulosis. Preliminary investigation of these two cases indicates that considerable similarities probably exist in the pathogenesis of malignant atrophic papulosis and the atropie blanche lesions of systemic lupus erythematosus. It is concluded that a diagnosis of malignant atrophic papulosis should only be made after systemic lupus erythematosus has been excluded by full investigation.

The cutaneous lesions of malignant atrophic papulosis (Degos' disease) are usually so distinctive that the diagnosis can often be made 'at a glance' by an experienced observer. Recently, Dubin & Stawiski (1974) described a patient with systemic lupus erythematosus in whom some of the papules evolved into lesions which were indistinguishable from those found in malignant atrophic papulosis. We report two further patients with systemic lupus erythematosus who, in the course of their disease, also developed atrophic papules which closely resembled those seen in malignant atrophic papulosis.

CASE REPORTS

Case 1. Mrs E.A. a 39-year-old Caucasian female. In March 1973 she first developed erythematous infiltrated lesions, about 1–2 cm in diameter, on her cheeks, nose, forehead and dorsa of hands. At that time the ANF was positive at a titre of 1 in 10 but no LE cells were found, WBC 3300/mm^3 and the ESR 14 mm in 1 h. A biopsy from one of the lesions on the face showed the histological changes of lupus erythematosus, and direct immunofluorescence confirmed the presence of granular deposits of IgG and IgM in the basement membrane zone (BMZ). Chloroquine 250 mg daily was started but soon had to be discontinued because of persistent nausea. By October 1973 most of the original infiltrated lesions had developed atrophic white centres. A number of the smaller papules, particularly on the trunk, also developed porcelain white centres with telangiectasia of their margins (Fig. 1) and were indistinguishable from the lesions of malignant atrophic papulosis. Biopsy of an early papule showed a marked accumulation of mucin in the centre of the lesion. In the later lesions the epidermis was atrophic with hyalinization and relative acellularity of the upper dermal collagen (Fig. 2). A moderately dense lymphocytic infiltrate was present around small blood vessels, particularly at the edges of the papule. Direct immunofluorescence of both lesional and clinically uninvolved skin showed a thready granular BMZ fluorescence with IgM, IgG and C3 (Fig. 3). In May 1974



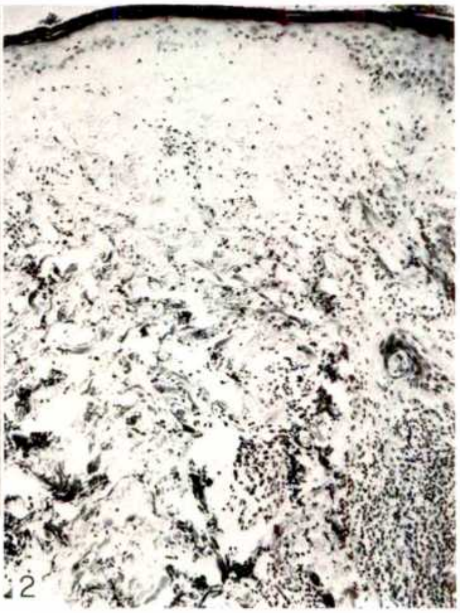


FIGURE 1. Case 1. Close up of lesion resembling malignant atrophic papulosis on trunk.
FIGURE 2. Case 1. Atrophy of the epidermis in the centre of the papule with hyalinization and relative acellularity of the upper dermis (H & E, × 100).

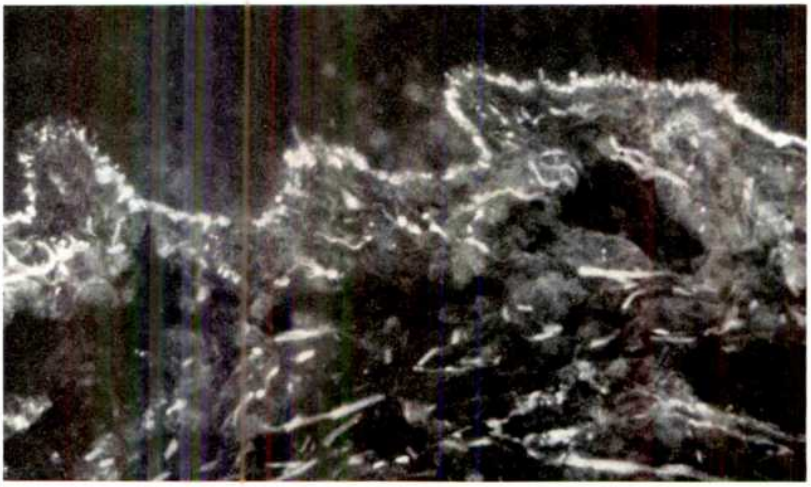


FIGURE 3. Case 1. Granular fluorescence in BMZ with IgG in a biopsy from clinically uninvolved skin.

treatment with mepacrine 100 mg daily was begun but larger painful atrophie blanche lesions appeared on the heels, dorsa of toes and the left ring finger. In January 1975 the mepacrine was stopped and the patient was admitted for reassessment. The atrophie blanche lesions had by then resolved but she felt generally unwell. She complained of persistent headaches and fleeting joint pains. About 50 small Degos-like lesions were present on her trunk and limbs and new papules were continuing to appear. By now the ANF was positive to a titre of 1 in 50 but no LE cells could be demonstrated. There was no proteinuria and the creatinine clearance was 92·5 ml/min. The euglobulin lysis time was $2\frac{3}{4}$ h (control 3 h). Fibrinolytic therapy (phenformin 50 mg twice daily and ethylestrenol 2 mg 4 times daily) was commenced but also had to be discontinued because of gastric intolerance. Prednisone 20 mg daily was begun and the patient rapidly improved and felt well. She has now been maintained

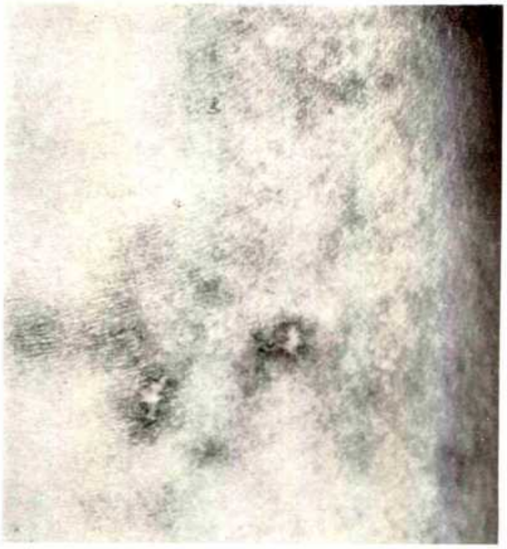


FIGURE 4. Case 2. Livedo reticularis over upper arm with two small atrophie blanche lesions resembling malignant atrophic papulosis.

on 12–15 mg prednisone daily for the past 12 months. The number of new papules appearing has been considerably reduced.

Case 2. Mrs J.E. a 37-year-old Caucasian female. This patient presented with typical discoid lupus erythematosus on her left cheek in 1966. The lesions were treated with betamethasone valerate cream (0·1%) but flared up during the summer months. She felt well and general examination was negative. ESR was 8 mm/h, ANF negative. In 1967 the more persistent lesions were treated with intra-lesional triamcinolone acetonide, but this resulted in atrophy. Treatment was started with chloroquine 200 mg daily but in 1968 she began to develop erythematous infiltrated lesions on her arms. Unfortunately the patient defaulted from follow up and was not seen again until March 1974. She had a reticulate livedo over the upper arms and in some areas central atrophy had occurred (Fig. 4) resembling malignant atrophic papulosis. Splinter haemorrhages were also noted under some finger nails. Investigations revealed haemoglobin 13·4 g%, WBC 9800 mm^3; ESR 12 mm/h; ANF positive to a titre of 1 in 80; LE cells present. Anti-DNA antibodies positive 1:40; DNA binding 70% (normal range 0–30%). A skin biopsy confirmed the diagnosis of lupus erythematosus and direct immunofluorescence from a lesion showed a thready BMZ fluorescence with IgM and IgG. Later the patient became unwell and developed alopecia, arthralgia and muscle weakness.

DISCUSSION

In both of our cases, but especially in Case 1, many of the erythematous papular or infiltrated lesions evolved into areas of atrophie blanche. Atrophie blanche has recently been reported in association with systemic lupus erythematosus and systemic sclerosis (Stevanovic, 1974) and results from partial or complete occlusion of dermal blood vessels due to fibrinoid deposition. That in our two cases, and the case described by Dublin and Stawiski (1974), many of the papules became atrophic (small atrophie blanche lesions) and closely resembled those seen in malignant atrophic papulosis (Degos' disease) is of extreme interest. Hitherto, malignant atrophic papulosis has been considered to be so distinctive that it has been suggested that a special histopathogenesis is implicated (Black, Nishioka & Levene, 1973).

Biopsies from the early papules in Case 1 showed considerable accumulation of mucin, a feature which has been previously noted in systemic lupus erythematosus (Gold, 1954; Potter & Handler 1964) but also occurs in malignant atrophic papulosis (Feuerman, Dollberg & Salvador, 1970;

Black, 1971; Black *et al.*, 1973). In the later atrophic lesions the histological features were also very similar to those that have been described in malignant atrophic papulosis.

Cytoplasmic inclusions (tubular aggregates) are regularly found within endothelial cells in systemic lupus erythematosus and some authors have suggested that the presence of these structures may be of diagnostic value (e.g. Prunieras *et al.*, 1972; Haustein, 1973). Cytoplasmic inclusions are also found within endothelial cells in malignant atrophic papulosis (Howard & Nishida, 1969; Muller & Landry, 1974) and it has been postulated that they may be of viral origin. Recently, however, Eady & Odland (1975) have shown that cytoplasmic inclusions occur in healing wounds and have suggested that these structures are not viral and are probably a product of regenerating endothelium. Considerable caution should therefore be applied before accepting that the demonstration of cytoplasmic inclusions in either malignant atrophic papulosis or systemic lupus erythematosus are indicative of a viral hypothesis.

Nevertheless, our preliminary investigations in these two cases indicate that considerable similarities exist in the pathogenesis of malignant atrophic papulosis and the atrophie blanche lesions that occur in systemic lupus erythematosus. It is likely that the wedge-shaped involvement of small blood vessels could arise from the slow occlusion of arterioles situated at a deeper level. Impairment of fibrinolytic activity in the centre of the papules has been observed in malignant atrophic papulosis (Black *et al.*, 1973) and Delaney & Black (1975) have reported the beneficial effect of fibrinolytic therapy in one patient with malignant atrophic papulosis. We were hoping to treat Case 1 with fibrinolytic therapy but unfortunately the patient was unable to tolerate the phenformin and ethylestrenol.

As a result of our experience with these two cases we agree with the conclusions of Dubin & Stawiski (1974) that a diagnosis of malignant atrophic papulosis should only be made after other collagen diseases, in particular systemic lupus erythematosus, but also possibly systemic sclerosis and mixed connective tissue disease, have been excluded by full investigation. The role of fibrinolytic enhancement in the treatment of malignant atrophic papulosis and atrophie blanche is worthy of further investigation.

REFERENCES

BLACK, M.M. (1971) Malignant atrophic papulosis. *British Journal of Dermatology*, **85**, 290.

BLACK, M.M., NISHIOKA, K. & LEVENE, G.M. (1973) The role of dermal blood vessels in the pathogenesis of malignant atrophic papulosis (Degos' disease): A study of two cases using enzyme histochemical, fibrinolytic, electronmicroscopical and immunological techniques. *British Journal of Dermatology*, **88**, 213.

DELANEY, T.J. & BLACK, M.M. (1975) Effect of fibrinolytic treatment in malignant atrophic papulosis. *British Medical Journal*, **iii**, 415.

DUBIN, H.V. & STAWISKI, M.A. (1974) Systemic lupus erythematosus resembling malignant atrophic papulosis. *Archives of Internal Medicine*, **134**, 321.

EADY, R.A.J. & ODLAND, G.F. (1975) Intra-endothelial tubular aggregates in experimental wounds. *British Journal of Dermatology*, **93**, 165.

FEUERMAN, E.J., DOLLBERG, L. & SALVADOR, O. (1970) Malignant atrophic papulosis with mucin in the dermis. *Archives of Pathology*, **32**, 189.

GOLD S.C. (1954) An unusual papular eruption associated with lupus erythematosus. *British Journal of Dermatology*, **66**, 429.

HAUSTEIN, U.-F. (1973) Tubular structures in affected and normal skin in chronic discoid and systemic lupus erythematosus: electron microscopic studies. *British Journal of Dermatology*, **89**, 1.

HOWARD, R.O. & NISHIDA, S. (1969) A case of Degos' disease with electron-microscopic findings. *Transactions of the American Academy of Ophthalmology and Otolaryngology*, **73**, 1097.

MULLER, S.A. & LANDRY, M. (1974) Exchange autografts in malignant atrophic papulosis (Degos' disease). *Mayo Clinic Proceedings*, **49**, 884.

POTTER, R. & HANDLER, R. (1964) Systemic lupus erythematosus with papular mucinosis. *Archives of Dermatology*, **90**, 446.

PRUNIERAS, M., GRUPPER, C., DUREPAIRE, R., EISEMANN, D. & REGNIER, M. (1972) Les inclusions type lupus dans la peau. *La Nouvelle Presse Medicale*, **1**, 1133.

STEVANOVIC, D.V. (1947) Atrophie blanche: A sign of dermal blood vessel occlusion. *Archives of Dermatology*, **109**, 858.

British Journal of Dermatology (1976) **95**, 653.

Adverse skin reaction from vitamin K1

H.M.BARNES AND I.SARKANY

Department of Dermatology, Royal Free Hospital, London NW3

Accepted for publication 10 May 1976

SUMMARY

Two patients with erythematous, tender, indurated plaques at the site of intramuscular injections of vitamin K1 are described. In one of these, the condition was reproduced by the injection of a test dose. In both cases, there was associated liver disease. The red, indurated plaques cleared in 4–14 days.

There have been two previous reports in the literature of similar lesions, although some of the patients showed sclerodermatous changes at the same site within 2 years of the injections. These unusual reactions have only been seen in patients with liver disease and when a variety of drugs have been administered. It may be that these factors are important in the initiation of the cutaneous response.

Severe local reactions due to intramuscular injections of vitamins are rare and the few cases reported do not conform to a uniform pattern. This is a report of two patients with liver disease who developed localized red, tender, infiltrated plaques 4–14 days after intramuscular injections of vitamin K1. The lesions persisted for 2 months but settled without leaving permanent scarring.

CASE REPORTS

Case 1

A 31-year-old woman, investigated in 1974 for secondary infertility, was found to have polycythaemia rubra vera and hepatomegaly. A liver biopsy suggested venous outflow obstruction and Budd Chiari syndrome was diagnosed. She was given intramuscular injections of 10 mg vitamin K1 daily over several weeks. She was also treated with injections of amylobarbitone and atropine 1 month and 3 weeks before the reaction here described developed. She also had pethidine and promethazine on one occasion 2 weeks before the episode. She was taking several other drugs orally.

She developed an erythematous itchy infiltrated area about 10 × 10 cm on each buttock at the sites of the injections. An intradermal test injection of vitamin K1 into the forearm, produced an infiltrated erythematous area at the site of this injection 96 h later, as well as an exacerbation of the lesions on the buttocks, and a widespread transient maculo-papular erythematous eruption over the trunk. The infiltrated plaques on the buttocks took 2 months to settle completely but left no residual scarring.

Case 2

A 30-year-old woman was referred to the Medical Unit at the Royal Free Hospital from another hospital after taking an overdose of 25–50 paracetamol tablets. Nineteen hours after ingestion of the tablets she became jaundiced and several days later developed acute renal failure. She was treated with peritoneal dialysis and given intramuscular injections of 20 mg vitamin K1 daily for 5 days. Metoclopramide and pentazocine were also given intramuscularly over several days at the same time as the vitamin K1.

Two weeks after the last injection she developed localized infiltrated erythematous areas about 8 × 8 cm on both upper arms and thighs at the sites of the injections (Fig. 1). Over the next few days a widespread erythematous maculo-papular eruption appeared affecting the abdomen and limbs. The localized reactions at the sites of previous injections were erythematous, tender and indurated and the skin surface resembled orange peel. A biopsy was taken from one of the indurated lesions on the thigh. Histologically, this showed parakeratosis and spongiotic vesicles within the epidermis. There was patchy infiltration in the dermis by eosinophils and polymorphonuclear leukocytes, and a marked perivascular reaction consisting mainly of eosinophils and histiocytes.

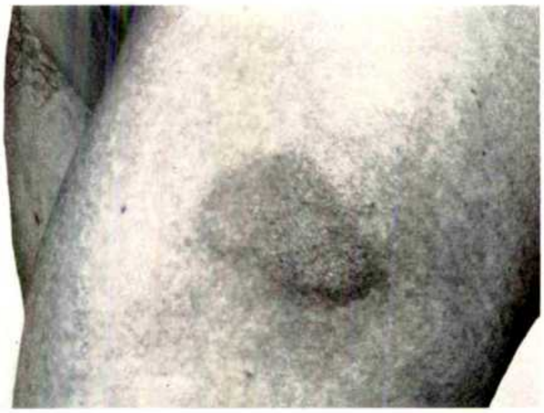


FIGURE 1. Upper outer thigh showing infiltrated plaque at the site of intramuscular injection of vitamin K1.

The generalized rash on the trunk and limbs settled within 10 days but the indurated areas on the thighs and arms took about 2 months to disappear. No detailed skin tests with vitamin K1 could be carried out subsequently, since the patient failed to re-attend the hospital.

DISCUSSION

Vitamin K1 (phytomenadione) is a naturally-occurring vitamin widely used specifically as an antidote to anticoagulants, and it is more generally employed in hypoprothrombinaemic states due to any cause. Other synthetic vitamin K preparations, particularly vitamins K3 and K4, are used in a similar way. Adverse effects from vitamin K1 are uncommon. The most important side effect of vitamin K1 is hyperbilirubinaemia and kernicterus in newborn infants, particularly in those that are premature (De Jonge, 1965; Roe, 1966). This complication is probably due to competition for glucuronyl transferase by the drug in the presence of immaturity of liver function (Wynn, 1963).

The administration of massive doses of vitamins K3 and K4 may cause haemolytic reactions (Rouquès, 1967) but this has not been described with vitamin K1. Vitamins K3 and K4 have also been reported to cause anaemia and pancytopenia (Lüdin, 1965), depression of prothrombin time, proteinuria, porphyrinuria, renal tubular degeneration and vomiting (Meyler & Herxheimer, 1963).

Allergic reactions to vitamin K1 are very rare but anaphylaxis and death have been reported after intravenous administration (Medical News, 1963; Pedersen, 1966) and the drug must therefore be administered very slowly when given intravenously. Urticaria developed in one patient following a single intramuscular dose of vitamin K1 (Carton, 1965).

Contact dermatitis from vitamin K4 was described in a woman working in a pharmaceutical establishment, who developed an eczematous eruption on the hands and face (Jirasek & Schwank, 1965). Patch tests were positive to vitamin K, and she was also found to be allergic to vitamin K4 (Menadiol) tablets and to an injectable solution of vitamin K4. Group sensitivity was demonstrated between Menadiol (vitamin K4) and Menadione (vitamin K3) but not with vitamin K1.

Our two patients developed severe localized reactions at the site of intramuscular injections of vitamin K1. The early changes resembled urticaria but were not transient and the swollen red plaques were caused by underlying dermal involvement as well as epidermal changes. Intradermal testing with vitamin K1 gave a positive result in the one case available for testing.

We have found two reports in the French literature of an allergic response to intramuscular vitamin K1 injection resembling our cases (Piguet & Bertheuil, 1964; Texier *et al.*, 1972). Two patients described by Piguet & Bertheuil developed large, hot, infiltrated erythematous plaques at the sites of vitamin K1 injection on the buttocks. In one case, there was also a widespread urticarial eruption. Intradermal tests with the vitamin K1 preparation gave positive results. Further tests with various constituents of the vitamin K1 preparation were performed. Patch tests were all negative. Intradermal tests with the oily vehicle, with vitamin K1 on its own and vitamin K1 mixed in olive oil were all negative. The tests with phenol and glycerine showed doubtful irritant positive results. It was felt that the various ingredients on their own were haptens. A subcutaneous injection gave a positive result only with the vitamin K preparation, which produced lesions similar to those seen on the buttocks.

In 1972, Texier *et al.* reported a similar eruption to that seen by Piguet & Bertheuil in nine cirrhotic patients, following intramuscular injections of vitamin K1. However, in their patients, regression of the active erythematous plaques was followed within 2 years by an atrophic sclerodermatous patch corresponding to the area covered by the original erythema. Histology of the early erythematous lesions showed a mixed inflammatory infiltrate with no eosinophils in the dermis, while the later patches showed a sclerodermatous histology, although this extended deeper and was more disorganized than normal scleroderma.

Intradermal testing with vitamin K1 was positive in four patients and lymphoblastic transformation in culture was demonstrated in one case. The authors of both reports wondered whether there was incompatibility between some of the drugs or whether the liver disease might have affected the response of their patients in producing the erythematous reaction, since tests of hepatic function, even in normal patients, may change after vitamin K1 administration. Vitamin K1 in large doses may produce hypoprothrombinaemia and bleeding in cases of liver disease (Smith & Custer, 1960).

Our two patients have shown no evidence of developing scleroderma at the site of the vitamin K1 injection but this could conceivably develop later, since they have not been followed for more than 1 year. Both our cases had liver disease and both were given a variety of intramuscular preparations in addition to the vitamin K1.

REFERENCES

CARTON, M.F.-X. (1965) Réaction allergique au cours d'un traitement: vitamine K1 + extrait de foie. *Bulletin de la Société française de dermatologie et de syphiligraphie*, **72**, 228.

DE JONGE, G.A. (1965) Vruchtbeschadiging door farmaca. *Folia Medica Neerlandica*, **8**, 65.

JIRASEK, VON L. & SCHWANK, R. (1965) Berufskontaktekzem durch Vitamin K. *Der Hautarzt*, **16**, 351.

LÜDIN, H. (1965) Blut- und Knochenmarkschädigungen durch Medikamente. *Schweizerische Medizinische Wochenschrift*, **95**, 1027.

MEDICAL NEWS (1963) Doctors warned on drug. *New York State Journal of Medicine*, **63**, 2430.

MEYLER, L. & HERXHEIMER, A. (eds.) (1963) *Side Effects of Drugs*, Vol. 4, p. 302. Excerpta Medica, Amsterdam.

PEDERSEN, J.Th. (1966) Allergiske Reaktioner efter Vitaminindgift. *Ugeskrift for Laeger*, **128**, 708.

PIGUET, MM.B. & BERTHEUIL, F. (1964) Accidents cutanés allergiques provoqués par une préparation injectable de vitamine K1 synthétique. *Bulletin de la Société française de dermatologie et de syphiligraphie*, **61**, 486.

ROE, D.A. (1966) Nutrient toxicity with excessive intake. 1. Vitamins. *New York State Journal of Medicine*, **66**, 869.

ROUQUÈS, L. (1967) Les thérapeutiques dangereuses chez les femmes enceintes. *La Presse Medicale*, **75**, 667.

SMITH, A.M. & CUSTER, R.P. (1960) Toxicity of vitamin K: induced hypoprothrombinaemia and altered liver function. *Journal of the American Medical Association*, **173**, 502.

TEXIER, L., GENDRE, Ph., GAUTHIER, O., GAUTHIER, Y., SURLÉVÉ-BAZEILLE, J-E. & BOINEAU, D. (1972) Hypodermites sclérodermiformes lombo-fessières induites par des injections médicamenteuses intramusculaires associées a la vitamine K1. *Annales de Dermatologie et de Syphiligraphie*, **99**, 363.

WYNN, R.M. (1963) The Obstetric significance of factors affecting the metabolism of bilirubin with particular reference to the role of vitamin K. *Obstetrical and Gynaecological Survey*, **18**, 333.

British Journal of Dermatology (1976) **95**, 657.

Congenital triangular alopecia

RAJ KUBBA AND ARTHUR ROOK*

Addenbrooke's Hospital, Cambridge

Accepted for publication 14 May 1976

SUMMARY

Three new cases of congenital triangular alopecia are reported and the differential diagnosis of this rare developmental defect is discussed.

Congenital triangular alopecia was first described by Sabouraud in 1905 in his '*Manuel élémentaire de Dermatologie topographique régionale*', which was subsequently published also in English translation. The condition was mentioned and illustrated in Sabouraud's (1932) well known book on disorders of the scalp and in such standard works of reference as Jadassohn's '*Handbuch*' (Galewsky, 1932), Darier's '*Nouvelle Pratique*' (Sabouraud, 1936) and McCarthy's (1940) '*Diseases of the Hair*'. Nevertheless fewer than ten cases have been reported. The fact that three new cases have been diagnosed in our department suggests that this interesting defect may often be overlooked or unidentified.

CASE REPORTS

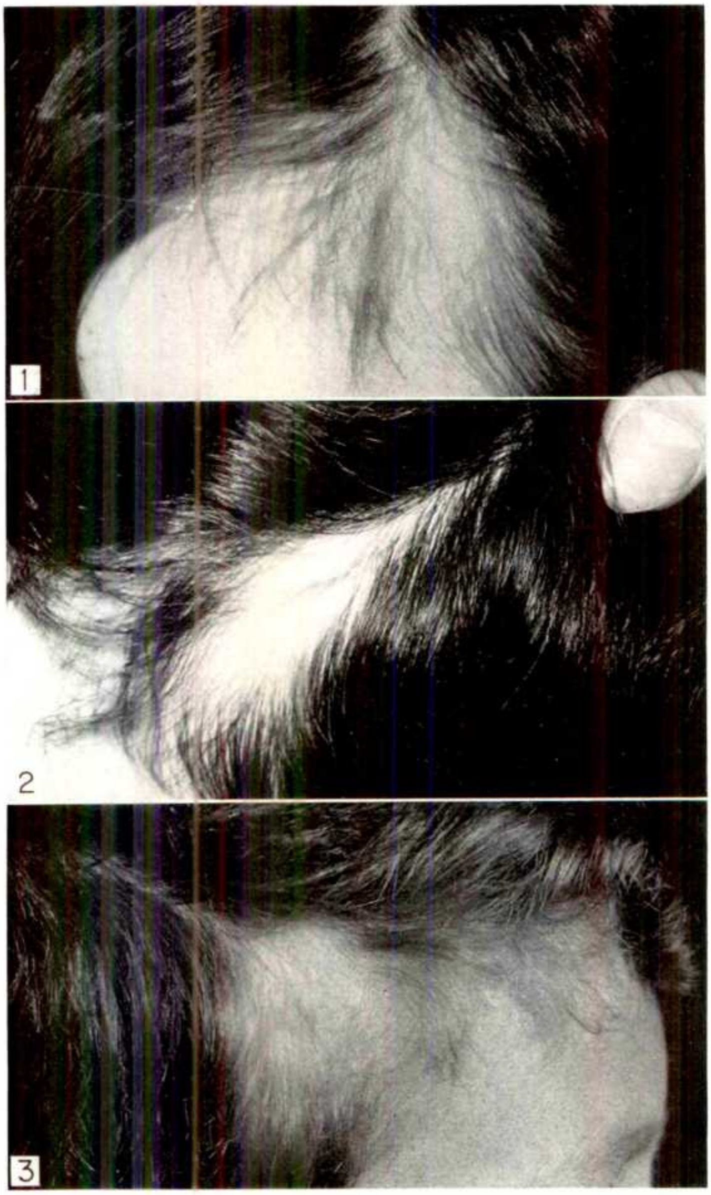
Case 1. J.A., a girl aged 4, was noticed at the age of 2 to have an area of alopecia in the left frontotemporal region. On examination (Fig. 1) there was a roughly triangular area of partial baldness with 4 cm sides and with its base extending towards but sparing the frontal hair line. A few normal hairs were still present in the bald area. There was no scarring. Eleven years later the parents reported that the condition was unchanged.

Case 2. G.A., a boy aged 5, was noticed recently to have developed a bald patch on the left side of his forehead. On examination (Fig. 2) he was found to have an irregularly triangular patch of non-scarring alopecia in the left frontotemporal region extending to the frontal hair line. A few normal hairs were present within the patch. He was lost to follow-up.

Case 3. R.S., a boy, was aged 3 when his parents became aware of a bald patch in the right frontotemporal region. A dome-shaped patch (Fig. 3) retained some terminal hair along its base at the frontal hair line, and a few fine vellus hairs at its centre. It was unchanged 9 years later.

---

* Requests for reprints should be addressed to Dr Rook.



FIGURES 1–3. Three cases of congenital triangular alopecia, showing the constant site and the variation in shape and extent.

DISCUSSION

Congenital triangular alopecia is usually unilateral but may be bilateral (Minars, 1974). The shape of the patch is only approximately triangular, with its base extending to and involving, or partially sparing the hair line. The only published histological report (Minars, 1974) showed no abnormality other than replacement of the normal abundant terminal follicles by sparse vellus follicles. The condition remains unchanged throughout life (Canizares, 1941; Minars, 1974).

There appears to be no obvious phylogenetic or embryological explanation for these lesions. They have once been reported in siblings (Degos & Rabut, 1949). In only one case report (Muller, 1973) of a child, who also had congenital dislocation of one hip, has any associated defect been mentioned. An interesting feature is the fact that in the majority of cases the area of alopecia is not noticed by the parents until the third year or later; not until the age of 5 in our second case. In some infants the hair is sparse and fine during the first year and the adult mosaic pattern of follicular activity may not be fully established until early in the second year (Pecoraro *et al.*, 1964). The failure of parents to become aware of the patch until it contrasts strikingly with the surrounding normal hair is understandable; that it could pass undetected to the age of 5 is perhaps improbable. It is possible that as in some other circumscribed alopecias of congenital origin (Friederich, 1949) the follicles are of normal size in the early post-natal hair cycles and that the defect may not in fact be manifest until some time after birth.

A condition which resembles congenital triangular alopecia in many respects, but which commonly involves the vertex and is said always to be apparent at birth, has been named 'aplastic naevus' by Schoenfeld & Mehregan (1974). A circumscribed but often irregularly shaped area is histologically normal apart from the absence of skin appendages (Meyer & Civatte, 1960). Why the alopecia is manifest so early in this condition and so late in triangular alopecia is a matter for speculation. Aplastic naevus must not be confused with aplasia cutis in which all layers of the skin are deficient.

Differential diagnosis (Huriez & Desmons, 1961) is seldom a problem provided the possibility of congenital triangular alopecia is considered. Sabouraud (1932) emphasized the occasional resemblance to alopecia areata, a diagnosis readily excluded by the course. The site may suggest a traumatic alopecia resulting from a hair-pulling tic, but careful examination will immediately exclude this diagnosis. The inconstant sutural alopecia of the Hallermann–Streiff syndrome is of later onset, more extensive and more dramatic. The many circumscribed cicatricial alopecias of childhood are readily eliminated, and will not be further discussed.

REFERENCES

CANIZARES, O. (1941) Alopecia triangularis congenitalis. Report of a case. *Archives of Dermatology and Syphilology*, **44**, 1106.

DEGOS, R. & RABUT, R. (1949) Alopécie angulaire congénitale de la tempe. *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **5**, 487.

FRIEDERICH, H.C. (1949) Zur Kenntnis des angeborenen umschriebenen Haarausfalles. *Dermatologische Wochenschrift*, **120**, 712.

GALEWSKY, E. (1932) In: *Handbuch der Haut- und Geschlechtskrankheiten* (Ed. by J. Jadassohn), Vol. 13, pt. 1, p. 193. Springer, Berlin.

HURIEZ, C. & DESMONS, F. (1961) Les alopécies circonscrites de l'enfant leur aspects cliniques et leur traitement. *Revue du Practicien*, **11**, 1927.

MCCARTHY, L.C. (1940) *Diagnosis and treatment of diseases of the hair*, p. 135. Mosby, St Louis.

MEYER, J. & CIVATTE, J. (1960) Alopécie congénitale du tourbillon. *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **67**, 457.

MINARS, N. (1974) Congenital temporal alopecia. *Archives of Dermatology*, **109**, 395.

MULLER, S.A. (1973) Syndromes of genetic significance. *Journal of Investigative Dermatology*, **60**, 475.

PECORARO, V., ASTORE, I., BARMAN, J. & ARANJO, C.I. (1964) The normal trichogram in the child before puberty. *Journal of Investigative Dermatology*, **42**, 427.

SABOURAUD, R. (1905) *Manuel élémentaire de Dermatologie topographique régionale*. Masson, Paris.

SABOURAUD, R. (1932) *Diagnostic et traitement des affections du cuir chevelu*. Masson, Paris.

SABOURAUD, R. (1936) In: *Nouvelle pratique dermatologique* (Ed. by J. Darier et al.) Vol. 8, p. 11. Masson, Paris.

SCHOENFELD, R.J. & MEHREGAN, A.H. (1974) 'Aplastic naevus', the 'minus' naevus. *Cutis*, **12**, 386.

British Journal of Dermatology (1976) **95**, 661.

Eosinophilic pustular folliculitis

REPORT OF A EUROPEAN CASE

ROLF HOLST

Department of Dermatology, University of Lund, Malmö General Hospital, S-124 01 Malmö, Sweden

Accepted for publication 25 May 1976

SUMMARY

A case of eosinophilic pustular folliculitis (Ofuji) is presented. This long-lasting disease of unknown aetiology has hitherto been reported only from Japan. It is characterized by tinea-like slowly growing rings and by blood eosinophilia. The histological changes are epidermal infiltrations and abscesses of predominantly eosinophilic leukocytes, often localized to the follicles.

Eosinophilic pustular folliculitis is a new disease entity, first described by Ofuji *et al.* (1970). Up to now there are 18 cases reported in the literature, all from Japan (Ishibashi *et al.* 1974).

CASE REPORT

A Caucasian male, born in 1943. He is a taxi driver but up to 1962 he was a sailor and as such he visited many distant countries (not Japan). He had always been healthy, when in 1965 he noticed an eruption on his face. After some months the eruption spread to the trunk and the soles. Except for moderate pruritus there were no symptoms. He was admitted to our clinic in September 1966. His general condition was good. On the face there was an asymmetrical, slightly infiltrated, irregular, reddish rash containing solitary small pustules, of which many seemed to be located in the follicles. On the soles there were sharply demarcated reddish plaques with large, non-tender pustules, much resembling palmo-plantar pustulosis. On the trunk there were tinea-like, rounded and polycyclic, slightly infiltrated rings with many small pustules (Figs 1 & 2). The rings were growing slowly peripherally (1–2 mm/week) and had a diameter of up to 30 cm. The active border was usually 1–2 cm broad and there was a tendency to central healing.

The diagnosis was for a long time not clear. Penicillin, griseofulvin and prednisolone were tried without any certain effect. There were relapses and remissions until March 1967, when he suddenly was free of his disease. In 1971 a new relapse of the same kind occurred. He was admitted to hospital for two periods in 1972 and 1973. Many treatments were tried again but only sulphones seemed to have some effect. Sulphapyridine had no effect. For the last few years the disease has been in a rather quiescent state, not influencing his daily activities.

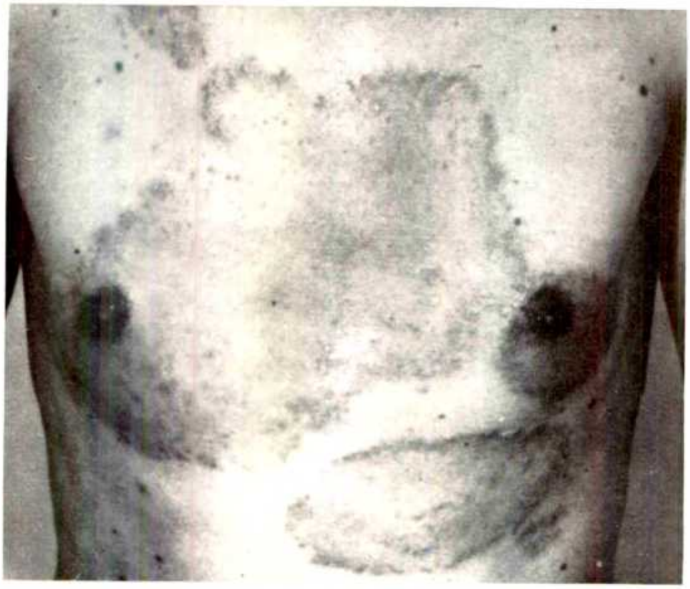


FIGURE 1. Annular infiltrates with many pustules and central clearing on the trunk.

Investigations. He has had a slight leucocytosis (up to 15000/mm^3) with 25–40% eosinophils (average total count 2800/mm^3). In a quiescent state of the disease there was only a slight eosinophilia (6% of 9300 w.b.c./mm^3). The bone marrow was normal except for eosinophilia.

The following investigations were normal: ESR, serum protein electrophoresis, serum creatinine, electrolytes, B_{12}, folic acid, antistreptolysin titre, antinuclear factor; X-ray of the heart and the lungs, and intravenous urography; and examinations for anal eggs. Repeated cultures from the pustules were negative both for bacteria and fungi. Intradermal tuberculin test was positive (expected in Sweden) and trichophytin test negative.

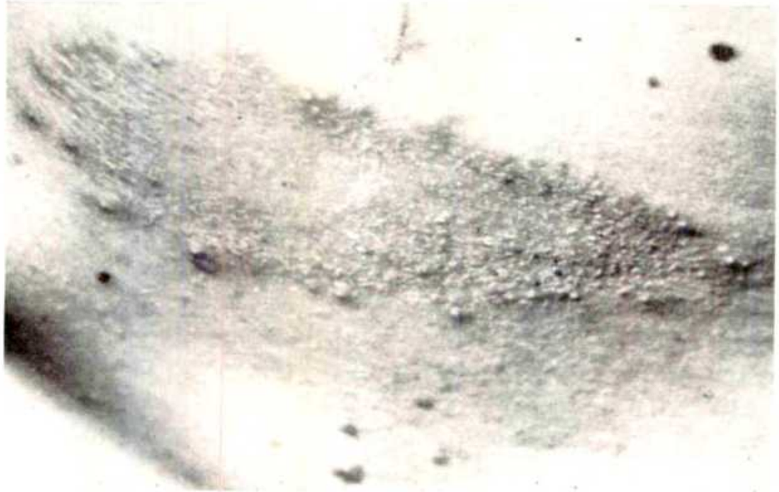
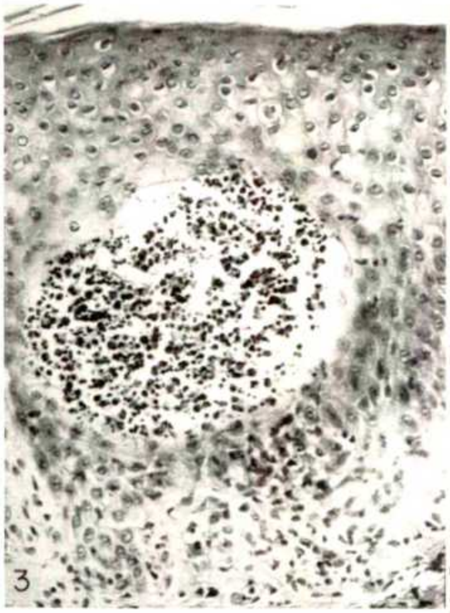


FIGURE 2. Close up view of an annular infiltration showing the arrangement of the pustules.



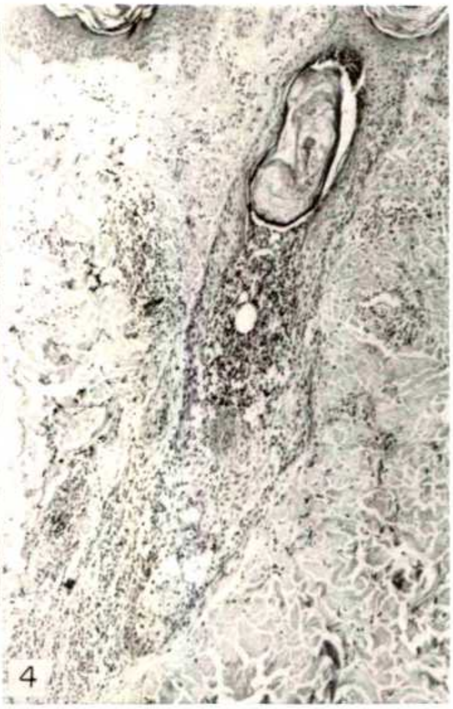


FIGURE 3. Intra-epidermal abscess filled with eosinophilic leukocytes (H & E, × 224).
FIGURE 4. Picture showing follicular infiltration of eosinophilic leukocytes (H & E, × 88).

Histopathology. In the epidermis there was an exocytosis with predominantly eosinophilic leukocytes. Where this infiltration was dense there was a 'secondary' spongiosis with abscesses situated at different levels of the epidermis. In the border of one of the abscesses there was a small spongiform area, but the other abscesses were all distinctly separated from the surrounding epidermis, that is they were not of the so called Kogoj type (Fig. 3). Most of these eosinophilic infiltrates were located in or near the follicles (Fig. 4). In a few areas there were small foci of acantholysis suggestive of pemphigus. In the dermis there were moderate perivascular infiltrates, even these consisting predominantly of eosinophilic leukocytes.

Immunofluorescence. The immunofluorescence of the lesions by the direct method (using anti-IgG, anti-IgM and anti-IgA) did not show any immunoglobulins between the epidermal cells or at the basement membrane. In the blood there were no circulating antibodies against the intercellular substance of the epidermis or against the basal membrane.

DISCUSSION

Our patient has a relapsing, mildly itching skin disease consisting of annular and polycyclic infiltrates with many pustules. There is no mucosal involvement and no general symptoms. Histologically there are predominantly follicular infiltrates of eosinophilic leukocytes giving rise to frequent epidermal abscesses, which are not of the spongiform type. There is a distinct blood eosinophilia. Bacteriological and mycological cultures are negative. There are no signs of intestinal parasites.

The first diagnosis discussed was a tinea infection. The negative cultures and unsuccessful griseofulvin treatment exclude this diagnosis. Another presumptive diagnosis was an atypical dermatitis herpetiformis. The clinical and histological picture, the lack of immunoglobulin A at the basement membrane, and the only slight effect of sulphones exclude this diagnosis.

The absence of spongiform pustules and the blood and skin eosinophilia, speak strongly against pustular psoriasis of the so called annular type (Degos, Civatte & Arrouy, 1966; Braun-Falco & Weidner, 1968).

The small areas of acantholysis found in one biopsy made us think of pemphigus as a possible differential diagnosis. The so-called herpetiform pemphigus has a protracted course and the lesions are erythematous and often gyrate or annular. Histologically there can be an eosinophilic spongiosis and there are often intra-epidermal pustules with many eosinophils (Jablonska *et al.*, 1975). The blood in these cases sometimes shows a distinct eosinophilia. However, there were no pemphigus antibodies in the blood of our patient and direct immunofluorescence examinations did not show any signs of immunoglobulins in the epidermis. The acantholysis found in some small areas is probably a result of proteolytic enzymes released from disintegrated leukocytes (Milne, 1972).

The clinical and histological picture fits well with the recently described eosinophilic pustular folliculitis (Ofuji *et al.*, 1970).

Unfortunately eosinophilic follicular dermatitis has no known aetiology but it seems to be a self-limiting disease and in the active periods antiphlogistic agents like corticosteroids and oxyphenbutazone are reported to be of considerable benefit (Ishibashi *et al.*, 1974).

REFERENCES

Braun-Falco, O. & Weidner, F. (1968) Psoriasis von Typ des Erythema annulare centrifugum mit Pustulation *Hautarzt*, **19**, 109.

Degos, R., Civatte, J. & Arrouy, M. (1966) Psoriasis et psoriasis pustuleux à type d'érythème annulaire centrifuge (3 cas). *Bulletin de la Société Française de Dermatologie et de Syphiligraphie*, **73**, 356.

Ishibashi, A., Nishiyama, Y., Miyata, C. & Chujo, T. (1974) Eosinophilic pustular folliculitis (Ofuji). *Dermatologica*, **149**, 240.

Jablonska, S., Chorzelski, T.P., Beutner, E.H. & Chorzelska, J. (1975) Herpetiform pemphigus. A variable pattern of pemphigus. *International Journal of Dermatology*, **14**, 353.

Milne, J.A. (1972) *An Introduction to the Diagnostic Histopathology of the Skin*, p. 44. Edward Arnold, London.

Ofuji, S., Ogino, A., Horio, T., Ohseko, T. & Uehara, M. (1970) Eosinophilic pustular folliculitis. *Acta dermato-venereologica*, **50**, 195.

British Journal of Dermatology (1976) **95**, 665.

Comment

LANGERHANS CELLS: INVOLVEMENT IN THE PATHOGENESIS OF MYCOSIS FUNGOIDES

Function of Langerhans cells

Langerhans cells (Lc) were initially described in 1868 (Langerhans, 1868) and since that time there has been much speculation concerning the origin, associations and functions of these clear cells, situated in the higher levels of the epidermis.

Although initially considered to be related to melanocytes (Billingham & Medawar, 1953) present evidence favours a mesodermal origin (Breathnach *et al.*, 1965; Wolff, 1972). It is now accepted that these dendritic cells form a relatively constant population admixed with epithelial cells in the stratified epithelia of the epidermis, oesophagus and cervix (Wolff & Winkelmann, 1967; Zelickson & Mottaz, 1968). In such sites, the cells appear to be capable of maintaining their position while the surrounding cells keratinize and are sloughed off. It has been suggested that they may be a form of epidermal scavenger (Hashimoto & Tarnowski, 1968). Unfortunately, the keratinocytes with which they form a close association are considerably more efficient at endocytosis of injected tracers (Wolff & Schreiner, 1970). Thus, if the Langerhans cell is a macrophage, it is of the poorly phagocytic variety.

Other suggestions concerning function include control of keratinization (Jarrett & Spearman, 1964) and control of keratinocyte proliferation (Potten & Allen, 1976). These theories either lack experimental corroboration or fail to account for all the known facts concerning the cell type.

It was Prunieras (1969) who first made the suggestion that antigens applied to the integument might interact with Langerhans cells. This speculation has recently been elegantly substantiated by Silberberg (1971), at the New York University School of Medicine. Such an observation is of fundamental importance in immunology, since it indicates that Langerhans cells may play an important role in the primary immune response. Shelley & Juhlin (1976) have confirmed the findings of the New York group.

Primary immune response

The evidence for a role of Langerhans cells in allergic contact hypersensitivity may be briefly summarized as follows. Close apposition (peripolesis) of mononuclear cells to Langerhans cells has been reported at the sites of positive allergic contact hypersensitivity reactions to mercuric chloride (Silberberg, 1971). These cell-to-cell interactions were not seen for doses in the contact primary irritant range. Langerhans cells also appear to be reduced in numbers and to exhibit signs of structural damage as a result of the allergic contact hypersensitivity reactions. These features seen with mercuric chloride have been noted also for other contact allergens (Silberberg *et al.*, 1975). Shelley & Juhlin (1976) applied nickel, cobaltous or chromic chloride hexahydrate as well as formaldehyde and glutaraldehyde to epidermal sheets of human and guinea pig skin. Subsequent histochemical staining for these allergens revealed their localization at sites corresponding to the dendritic Langerhans cell populations. Although these studies were limited to the light microscope level and satisfactory identification of the cell type requires visualization of its characteristic granules at the ultrastructural level, they do reveal an affinity of contact sensitizing substances for dendritic cells. This is suggestive of a network of receptor cells for antigens or haptens in the epidermis (Shelley & Juhlin, 1976), and hasled the authors to coin the term reticulo-epithelial system. The similarities to the reticulum cells of the lymph nodes of the reticulo-endothelial system are obvious and suggest the existence of systems for clearing antigen both from the integument and from the circulation. Since the epidermis and indeed other stratified epithelia are in the front line of exposure to external allergens it is surprising that the existence of such a sensory system has remained unremarked upon for so long. Once the presence of the sensory cells has been demonstrated many aspects of the primary immune response become clearer.

Silberberg and associates have carried out a variety of experiments to verify the initial hypothesis. Guinea pigs passively sensitized to DNCB by the transfer of mononuclear cells from actively sensitized animals also show the cell apposition and cellular damage typical of the earlier experiments (Silberberg, 1973). In addition, circulating Langerhans cells were noted in the lumen of dermal vessels in such situations (Silberberg *et al.*, 1976).

The observation of Langerhans cells in extra-epidermal sites in adoptive immune reactions is not the first report of such localizations. It is now accepted that Langerhans cells may be found in the dermis in a variety of normal and pathological conditions of the skin (Vernon *et al.*, 1973). The initial reports of Basset and co-workers concerning the presence of Langerhans cells in Histiocytosis X (Basset & Turiaf, 1965) have been confirmed for the complete spectrum of diseases of this entity (Morales *et al.*, 1969; Cancilla *et al.*, 1967). Until recently, however, it has been difficult to reconcile the appearance of Langerhans cells in lymph nodes, whether normal or abnormal (Kondo, 1969; Shamoto *et al.*, 1971), with the proposed functions of the cells.

The obvious step was taken by Silberberg (Silberberg *et al.*, 1976). Application of an antigen that is recognizable in the electron microscope such as ferritin, led to the observation of the label on the surface of Langerhans cells both within the epidermis and even more significantly, within the local lymph nodes. The suggestion may be made, therefore, that the Langerhans cell not only may act as the initial receptor in the integument, but also may be capable of transporting allergens to the local lymph nodes to initiate the primary immune response. Production in the lymph nodes of specifically sensitized lymphocytes capable of recognizing the allergens may thus lead to a 'homing' of such cells to the Langerhans cells in the epidermis. It is, therefore, predictable that the interaction of sensitized lymphocytes with the cell bearing the specific sensitizing substance might result in the generation of lymphokines. Both direct contact and lymphokines are capable of inducing cytotoxic changes in the target cells.

Mycosis fungoides

While the foregoing clearly points to the involvement of Langerhans cells in allergic contact hypersensitivity reactions, there is now the possibility of re-examining their involvement in a number of disease states involving the skin, especially those primarily associated with the epidermis.

Mycosis fungoides is an indolent infiltrative condition and it is typified by abundant reactive mononuclear cells in the superficial dermis and epidermis. The Pautrier micro-abscesses resulting from the penetration of the characteristic Lutzner or mycosis cells into the epidermis are one of the important diagnostic features. The Lutzner cell is no longer considered pathognomonic for mycosis fungoides, since similar cells have been noted in a range of non-lymphomatous diseases such as lupus erythematosus, lichen planus and psoriasis. Recent reviews of the nature of the cellular infiltrate point to a T cell lineage for the lymphocytic cells (Mackie *et al.*, 1976; Nordqvist & Kinney, 1976), although there is clearly a histiocytic element present in the dermal infiltrate (Goos *et al.*, 1976). Tan and associates (1974) have commented on these findings and suggested that mycosis fungoides is an example of antigen persistence resulting in the homing of specific reactive lymphocytes to the skin. This phenomenon, termed ecotaxis, has been the subject of a recent hypothesis in the Lancet by Goudie and associates (1974). Continuous stimulation of the immune system by antigens may ultimately lead to the development of imbalances and inappropriate responses. Auto-immune phenomena and even malignancy are the predictable results of such situations (Hobbs, 1968). The development of malignant clone(s) as a result of the chronic antigenic stimulation may, therefore, eventually lead to local lymph node involvement by a lymphomatous neoplastic cell type and subsequently to widespread dissemination. At this late stage it may be difficult or even impossible to separate the lymphoma associated with mycosis fungoides from other lymphomas originating in visceral lymphoid organs. The distinguishing features are, however, that the skin is primarily involved in mycosis fungoides rather than secondarily in the other lymphomas.

In reviewing the literature, and there has been a considerable effort to analyse the ultrastructure of mycosis fungoides (Rosas-Uribe *et al.*, 1974; Brownlee & Murad, 1970), there is little or no mention of Langerhans cells. Indeed, the major focus of attention has been placed on the cells in the dermis, both in terms of ultrastructure and more latterly in respect to their immunological characterization. Ryan and co-workers (1973) have, however, made specific mention of Langerhans cells in respect to mycosis fungoides. They suggest that the Langerhans cell may be the cell of origin of the mycosis cells seen in the condition. There is, however, little support for such an assertion, since no reports have been published of Langerhans granules in the infiltrating cells.

In recent observations of material from Stage II and III mycosis fungoides patients, we have paid special attention to the epidermal involvement and development of the so called micro-abscesses. Mononuclear cells infiltrating across the basal lamina of the epidermis were a common observation. These cells have the cerebriform nuclear profiles with elevated nuclear contour ratios (Shreck, 1972) typical of mycosis cells. Infiltration into the epidermis does not appear to induce immediately the production of micro-abscesses, since many infiltrating cells may be detected with no apparent ultrastructural damage observable in surrounding keratinocytes. Thus a spectrum of change may be noted from initial infiltration to disruption of the normal keratinocyte cell-to-cell contacts and production of cystic spaces containing mononuclear cells, inflammatory cells and cell debris. Emperipolesis of lymphoid cells within keratinocytes has been noted in the micro-abscesses. Interaction between cells falling within the definition of peripolesis is common in Stage II mycosis fungoides. The mycosis cells form close associations with keratinocytes, but more significantly, penetrating cells were frequently noted in the vicinity and in close association with epidermal Langerhans cells (Fig. 1). The identity of the Langerhans cells was verified in all cases by the presence of specific granules. The Langerhans cells also showed a variety of ultrastructural changes reminiscent of cytotoxic damage. These modifications include the development of bizarre nuclear shapes (Fig. 2).

These observations led us to propose the following hypothesis concerning the pathogenesis of mycosis fungoides. Since the condition has been shown in the early stages to simulate nonspecific dermatitic reactions, it may result from a chronic exposure to contact allergens. The nature of the persisting antigen is unknown and indeed it is probable that a variety of allergens may be involved. The persistence of the allergen could result from chronic environmental exposure or from a failure to clear the material from the epidermis. Clearly, if the nature of the allergens were known, it might be possible to interrupt the progression of the disease by appropriate prophylactic measures. At this stage, speculation concerning the possible candidates for the sensitizing agent is perhaps unwise. However, a recent report by Lyampert and associates (1976) of the existence of a cross-reactive antigen of thymus and skin epithelial cells common with the polysaccharide of group A streptococci is intriguing in that not only does it explain the autoimmune thymitis characteristic of rheumatic fever and other autoimmune processes, but may also have application to mycosis fungoides. This observation opens up several avenues for further investigation.

The recent observations on serum immunoglobulin levels in mycosis fungoides patients are also interesting. Mean levels of IgG, M, and D are not significantly different from control values (Mackie *et al.*, 1976; Tan *et al.*, 1974). Some indications of raised IgA levels have been noted (Mackie *et al.*, 1976; Robinowitz *et al.*, 1976), but even more significantly elevated levels of IgE have been reported (Mackie *et al.*, 1976; Tan *et al.*, 1974) in patients that were not atopic.

Bearing in mind the foregoing review of the function of Langerhans cells, it is suggested that here as in contact allergic hypersensitivity, the Langerhans cell acts as a trap for the externally applied allergen(s). By their capacity to transport material to the lymph nodes they may initiate the primary immune response, thereby inducing the formation of specifically sensitized lymphocytes. It is inter-

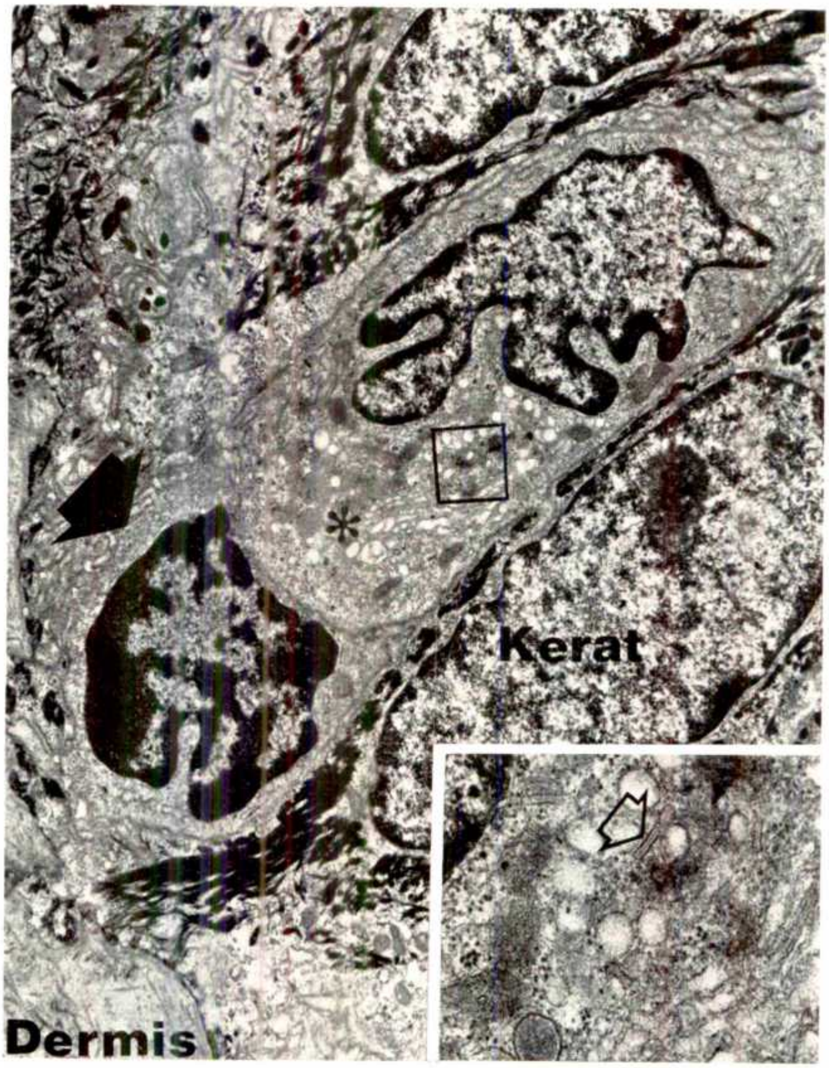


FIGURE 1. Epidermo–dermal junction. Infiltrating lymphocyte (arrow) closely apposed to a Langerhans cell (*). Adjacent keratinocytes show normal nuclei and keratin filaments (× 10,400). Insert. Higher magnification of boxed area. Langerhans cell granule (arrow) (× 42,800).

esting that lymphocytes transformed *in vitro* with PHA have several ultrastructural similarities with the cells seen in the infiltrate of mycosis fungoides. It remains then for these transformed cells to home to the sites of initial allergen fixation in the epidermis. The reactive cells are capable of infiltrating into the epidermis eventually to contact the allergen presented on the surface of the Langerhans cells. Lymphocytotoxic mechanisms involving direct contact or secretion of diffusible lymphokines would once initiated lead to various degrees of specific and non-specific damage to Langerhans cells and surrounding keratinocytes. It is interesting that another condition in which micro-abscesses are found in the epidermis is in certain dermatophytoses involving penetration of fungal hyphae (Graham *et al.*, 1972). It may not be too wild a suggestion to consider such intrusions into the integument as sources of chronic antigenic stimulation.

Other lymphokines such as migration inhibition factor (MIF) produced by the specifically sen-

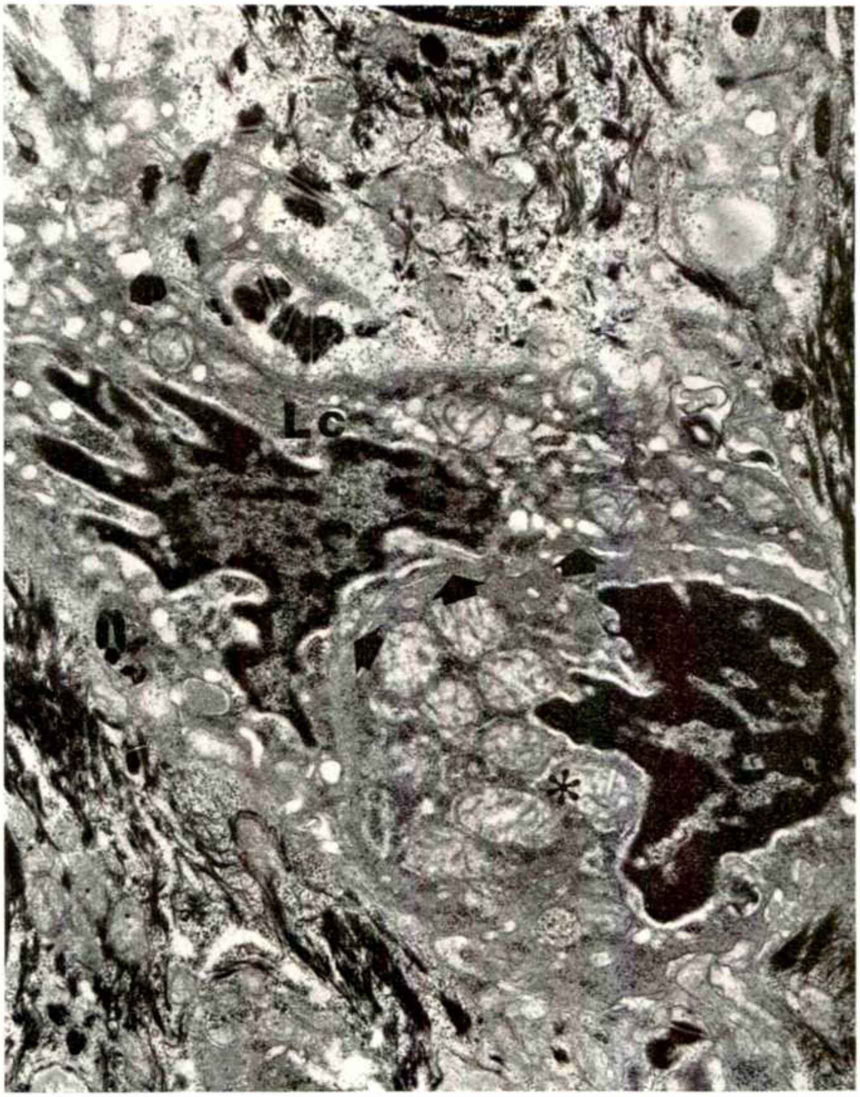


FIGURE 2. Infiltrating lymphocyte with irregular nuclear profile (*) closely apposed (arrows) to a Langerhans cell (Lc). Epidermis of a Stage II mycosis fungoides specimen (× 23,600).

sitized lymphocytes might also lead to the accumulation of histiocytes in the area of reaction. Elevated levels of MIF have been noted in the circulation of patients with Sézary syndrome (Yoshida *et al.*, 1975), a condition which some authors consider the leukaemic phase of mycosis fungoides.

Another example of prolonged antigenic stimulation leading to a malignant change in the lymphoreticular system can be seen in Tropical Splenomegaly or Big Spleen Disease where a small number of individuals terminate their supposed continued responsiveness to malarial antigens with a leukaemic condition (Anand & Davey, 1965).

The concept of immune derangement accompanying persistent antigenic stimulation, in malignancy, has been suggested and this may be indicated by a triad of immune complexes, anti-immunoglobulin antibodies and selective cellular immune unresponsiveness or anergy (Lewis *et al.*, 1976; Jerry *et al.*, 1976). Preliminary studies in this laboratory have demonstrated such a triad, in a small number of individuals with mycosis fungoides, and suggest that this disorder shows common features of immune derangement, associated with chronic antigenic stimulation. Fig. 3 illustrates the role of Langerhans cells in contact allergic hypersensitivity reactions and some possible parallels in mycosis fungoides.

If this hypothesis is to be acceptable, it is necessary to explain why such a reaction is not the rule rather than the exception. Clearly the fact that most individuals with mycosis fungoides are elderly may indicate an increased potential for an immune derangement of whatever form, with age. The suggestion that mycosis fungoides results from a breakdown in host immune surveillance is not a new one but it is one that would account for the sequential development from non-specific reactive dermatitis to malignant lymphoma. The recent attempts to characterize immunologically mycosis cells using cell surface marker methods as well as by tests such as the mixed leukocyte culture (MLC) and mitogen responsiveness (Edelson *et al.*, 1974) should provide important insights into the possible nature of the immune imbalance. Among the candidates for such a derangement are those involving suppressor cell function. Clearly the infiltrate in mycosis fungoides needs to be analysed with this aspect of immune regulation in mind.

Besides these possibilities, it may be that the defect lies in the Langerhans cell itself. It is probable that the Langerhans cell in normal epithelia is capable of clearing allergens from its surface in order not to present a continuous source of stimulation. Here again it is interesting that the peculiar granules so characteristic of the cell have as yet had no satisfactory function allocated to

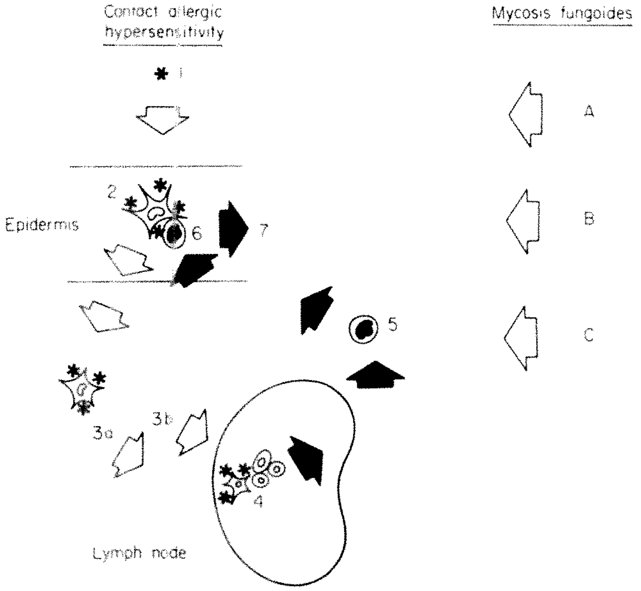


FIGURE 3. Diagram illustrating involvement of Langerhans cells in contact allergic reactions and possible relationships in mycosis fungoides.

KEY. 1. Exogenous allergen, 2. Attachment to Lc, 3. Transport to local lymph node, 3a. via Lc, 3b. Transfer to macrophage, 4. Stimulation of production of specifically sensitized lymphocytes, 5. Homing of reactive lymphocytes to the skin, 6. Infiltration into epidermis and peripolesis to Lc, 7. Production of lymphokines—lymphocytotoxins resulting in micro-abscess formation; MIF resulting in histiocyte accumulation in the dermis. A. Allergen persistence in the environment. B. Failure to clear allergen for Lc surface. C. Breakdown in immune regulation—Suppressor cells, etc.

them. Present evidence favours the suggestion that they fuse with the surface membrane and thus may be involved in regulating the surface properties of the cell. No evidence for a secreted substance has yet been produced. The granule has a characteristic lattice-like core, initially in the mid-line between the closely apposed membranes and it is assumed that the material giving rise to this pattern must become part of the external plasma membrane when the granule fuses with it and turns inside out. It is possible that specific allergen receptor sites might be disposed along initially the inside of the granule and thence to the exterior surface of the cell.

Much further work remains to be carried out to determine the validity of these suggestions. Clearly an attempt to characterize the Langerhans cell granules must be one of the priorities as well as determining the normal pattern of allergen clearance from the cell surface. In this way it will be possible to determine if the defect leading to antigen persistence in mycosis fungoides is one involving the Langerhans cell or is associated with other parts of the immune sensory and effector systems.

The stimulus to research in a variety of pure and applied immunology fields made by Silberberg's findings with respect to Langerhans cells is difficult to gauge. What is certain is that a wealth of data about the cell type must now be looked at from a new viewpoint and it will hopefully result in significant insights into a variety of conditions involving the skin, as for example in lymphomatoid papulosis and actinic reticuloid.

G.ROWDEN
M.G.LEWIS

McGill University Cancer Research Unit,
McIntyre Medical Sciences Building,
3655 Drummond St,
Montreal,
Quebec H3G 1Y6,
Canada

REFERENCES

ANAND, S.U. & DAVEY, W.W. (1965) Surgery of the spleen in Nigeria. *British Journal of Surgery*, **52**, 335.

BASSET, F. & TURIAF, M.J. (1965) Identification par la microscopie electronique de particules de nature problement virale dans les liasons granulomateuses d'une histocytose 'X' pulmonaire. *C. R. Acad. Sci. (D) Paris*, **261**, 3701.

BILLINGHAM, R.E. & MEDAWAR, P.B. (1953) A study of the branched cells of the mammalian epidermis with special reference to the fate of their division products. *Philosophical Transactions of the Royal Society of London B*, **237**, 151.

BREATHNACH, A.S. (1965) The cell of Langerhans. *International Review of Cytology*, **18**, 1.

BREATHNACH, A.S., SILVERS, W.K., SMITH, J. & HEYNER, S. (1965) Langerhans cells in mouse skin experimentally deprived of its neural crest component. *Journal of Investigative Dermatology*, **50**, 147.

BROWNLEE, T.R. & MURAD, T.M. (1970) Ultrastructure of mycosis fungoides. *Cancer*, **26**, 686.

CANCILLA, P.A., LAHEY, M.E. & CARNES, W.H. (1967) Cutaneous lesion of Letterer-Siwe disease. Electron microscopic study. *Cancer*, **20**, 1986.

EDELSON, R.L., LUTZNER, M.A., KIRKPATRICK, C.H., SHEVACH, E. & GREEN, O. (1974) Morphologic and functional properties of atypical T lymphocytes of the Sézary syndrome. *Mayo Clinic Proceedings*, **49**, 558.

GOOS, M., KAISERLING, E. & LENNERT, K. (1976) Mycosis fungoides: Model for T lymphocyte homing to the skin. *British Journal of Dermatology*, **94**, 221.

GOUDIE, R.B., MACFARLANE, P.S. & LINDSAY, M.K. (1974) Homing of lymphocytes to non-lymphoid tissues. *Lancet*, **i**, 292.

HASHIMOTO, K. & TARNOWSKI, W.M. (1968) Some new aspects of the Langerhans cell. *Archives of Dermatology*, **97**, 450.

HOBBS, J.R. (1968) Immune imbalance in dysgammaglobulinaemia type IV. *Lancet*, **i**, 110.

JARRETT, A. & SPEARMAN, R.I.C. (1964) *Histochemistry of the skin: Psoriasis.* English University Press.

JERRY, L.M., LEWIS, M.G. & CANO, P. (1976) Anergy, anti-antibodies and immune complex disease: A syndrome of disordered immune regulation in human cancer. *Proceedings of the Laval Symposium on Immunocancerology*, in press.

KONDO, Y. (1969) Macrophages containing Langerhans cell granules in normal lymph nodes of the rabbit. *Zeitschrift für Zellforschung*, **98**, 605.

Langerhans, P. (1868) Über die Nerven der menschlichen Haut. *Virchow's Archives of Pathology and Anatomy*, **44**, 325.

Lewis, M.G., Hartmann, D.P. & Jerry, L.M. (1976) Antibodies and anti-antibodies in human malignancy: An expression of deranged immune regulation. *Annals of the New York Academy of Sciences*, **276**, 316.

Lyampert, I.M., Beletskaya, L.V., Borodiyuk, N.A., Gnezditskaya, E.V. Rassokhina, I.I. & Danilova, T.A. (1976) A cross-reactive antigen of thymus and skin epithelial cells common with the polysaccharide of group A Streptococci. *Immunology*, **31**, 47.

MacKie, R., Sless, F.R., Cochran, R. & de Sousa, M. (1976) Lymphocyte abnormalities in mycosis fungoides. *British Journal of Dermatology*, **94**, 173.

Morales, A.R., Fine, G., Horn, R.C. Jr., & Watson, J.H.L. (1969) Langerhans cells in a localized lesion of the eosinophilic granuloma type. *Laboratory Investigation*, **20**, 412.

Nordqvist, B., & Kinney, J.P. (1976) T and B cells and cell-mediated immunity in mycosis fungoides. *Cancer*, **37**, 714.

Potten, C.S. & Allen, R.D. (1976) Model implicating Langerhans cell in keratinocyte proliferation control. *Differentiation*, **5**, 43.

Prunieras, M. (1969) Special review article: Interactions between keratinocytes and dendritic cells. *Journal of Investigative Dermatology*, **52**, 1.

Robinowitz, B.N., Noguchi, S. & Roenigk, H.H. Jr (1976) Tumor cell characterization in mycosis fungoides. *Cancer*, **37**, 1747.

Graham, J.H., Johnson, W.C. & Helwig, E.B. (1972) *Dermal pathology*. Harper & Row, New York.

Rosas-Uribe, A., Variakojis, D., Molnar, Z. & Rappaport, H. (1974) Mycosis fungoides: An ultrastructural study. *Cancer*, **34**, 634.

Ryan, E.A., Sanderson, K.V., Bartak, P. & Samman, P.D. (1973) Can mycosis fungoides begin in the epidermis? A hypothesis. *British Journal of Dermatology*, **88**, 419.

Shamoto, M., Kaplan, C. & Katoh, A.K. (1971) Langerhans cell granules in human hyperplastic lymph nodes. *Archives of Pathology*, **92**, 46.

Shelley, W.B. & Juhlin, L. (1976) Langerhans cells form a reticulo-epithelial trap for external contact allergens. *Nature, London*, **261**, 46.

Shreck, R. (1972) Ultrastructure of blood lymphocytes from chronic lymphocyte leukemia and lymphosarcoma cell leukemia. *Journal of the National Cancer Institute*, **48**, 51.

Silberberg, I. (1971) Ultrastructural studies of Langerhans cells in contact sensitivity and primary irritant reactions to mercuric chloride. *Clinical Research*, **19**, 715.

Silberberg, I. (1971) Studies by electron microscopy on epidermis after topical application of mercuric chloride: Morphologic and histochemical findings in epidermal cells of human subjects who do not show allergic or primary intact reaction to mercuric chloride (0·1%). *Journal of Investigative Dermatology*, **56**, 147.

Silberberg, I. (1973) Apposition of mononuclear cells to Langerhans cells in contact allergic reactions. An ultrastructural study. *Acta dermato-venereologica*, **53**, 1.

Silberberg, I., Baer, R.L., Rosenthal, S.A., Thorbecke, G.J. & Berezowsky, V. (1975) Dermal and intravascular Langerhans cells at the sites of passively induced allergic contact sensitivity. *Cellular Immunology*, **18**, 435.

Silberberg, I., Baer, R.L. & Rosenthal, S.A. (1976) The role of Langerhans cells in allergic contact hypersensitivity: A review of findings in man and guinea pigs. *Journal of Investigative Dermatology*, **66**, 210.

Silberberg, I., Sinakin, I., Thorbecke, G.J., Baer, R.L., Rosenthal, S.A. & Berezowsky, V. (1976) Antigen bearing Langerhans cells in dermal lymphatics and in lymph nodes. (Abstract) *Cellular Immunology*, **25**, 137.

Tan, R.S.H., Butterworth, C.M., McLaughlin, H., Malka, S., & Samman, P.D. (1974) Mycosis fungoides—a disease of antigen persistence. *British Journal of Dermatology*, **91**, 607.

Vernon, M.L., Fountain, L., Krebs, H.M., Luiz, H.B., Fuccillo, D.A. & Sever, J.L. (1973) Birbeck granules (Langerhans cell granules) in human lymph nodes. *American Journal of Clinical Pathology*, **60**, 771.

Wolff, K. (1972) The Langerhans cell. *Current Problems in Dermatology*, Vol. 4 (Ed. by J.W.H. Mali), pp. 79–145. Karger, Basel.

Wolff, K. & Schreiner, E. (1970) Uptake, intracellular transport and degradation of exogenous protein by Langerhans cells. An electron microscopic-cytochemical study using peroxidase as tracer substance. *Journal of Investigative Dermatology*, **54**, 37.

Wolff, K. & Winkelmann, R.K. (1967) Ultrastructural localization of nucleoside triphosphatase in Langerhans cells. *Journal of Investigative Dermatology*, **48**, 50.

Yoshida, T., Edelson, R., Cohen, S. & Green, I. (1976) Migration inhibitory activity in serum and cell supernatants in patients with Sézary syndrome. *Journal of Immunology*, **114**, 915.

Zelickson, A.S. & Mottaz, J.H. (1968) Epidermal dendritic cells. A quantitative study. *Archives of Dermatology*, **98**, 652.

British Journal of Dermatology (1976) **95**, 673.

Brief Communications

Fibrinolytic agents in the treatment of Necrobiosis Lipoidica

E.L.RHODES *Department of Dermatology, St. Helier Hospital, Carshalton, Surrey*

Patients with cutaneous vasculitis are known to have decreased blood fibrinolytic activity and to respond to such fibrinolytic agents as phenformin and stanozolol (Cunliffe *et al.*, 1971). Angiopathy in diabetes as exemplified by diabetic retinitis is similarly associated with impaired fibrinolysis (Egeberg, 1963; Fearnley *et al.*, 1963; Almér *et al.*, 1975).

Diabetic angiopathy, as shown by thickening of the basement membrane and the deposition of glycoprotein in and around the blood vessel walls, is seen in both necrobiosis lipoidica and granuloma annulare (Handelsman *et al.*, 1962; McMillan *et al.*, 1966; Haim *et al.*, 1973). It was therefore decided to try fibrinolytic agents in patients with necrobiosis lipoidica, and stanozolol (Stromba) was used initially as it seemed the most effective fibrinolytic agent available. This produced a therapeutic response but in five cases the serum transaminase levels rose markedly. Thus, in view of reports of liver cancer with anabolic steroids (Johnson, 1975), the nicotinic acid derivatives inositol nicotinate (Hexopal) 1g three times a day and tetranicotinoyl fructose (Bradilan) 500 mg three times a day were used instead. Both these substances are active fibrinolytic agents (Kappert, 1961; Sommer, 1965; Gibelli *et al.*, 1966; Benaim *et al.*, 1975) and are apparently without severe side effects. Some patients, however, complain of flushing which was distressing enough in two patients to stop therapy but usually this symptom wore off after a month or two.

TABLE 1

Treatment group	Response to treatment			
	Static	Clearing	Cleared completely	Total
Stromba	1	2	0	3
Hexopal	0	9	1	10
Stromba then Hexopal	0	7	1	8
Bradilan	1	5	0	6
Stromba then Bradilan	2	1	0	3
Total	4	24	2	30

Thirty patients have so far been treated, two of these have cleared completely and 24 others have improved. One of the cases whose lesions cleared also had widespread lesions of granuloma annulare and these too disappeared while on treatment. Another patient with extensive granuloma annulare also noticed improvement on therapy. Of the two patients whose lesions cleared, one, a non-diabetic, had had the lesions for 20 years and the other, a diabetic, for 5 years—both patients having both shins extensively involved.

It should be emphasised that response to this type of therapy is slow and of the two patients whose lesions have cleared, one had been treated for 2½ years and the other for 9 months; the average time before improvement is noticed is about 6 months.

Fibrinolytic agents have been used in the past for the treatment of diabetic retinitis–Durabolin by Honerski (1974), salicylates by Carroll *et al.* (1972), heparin by Bozadzhieva *et al.* (1973) and dextran sulphate by Mine (1975) with some success. In view of the response of necrobiosis lipoidica to these nicotinic acid derivatives which are easy to give and apparently without harmful side effects it would seem important to use them in the treatment of diabetic vascular disorders.

We are concurrently investigating the fibrinolytic system of patients with necrobiosis lipoidica and granuloma annulare to see what effect these drugs have *in vitro*.

REFERENCES

ALMÉR L.O., PANDOLFI, M. & NILSSON I.M. (1975) Diabetic retinopathy and the fibrinolytic system. *Diabetes*, **24**, 529.

BENAIM, M.E. & DEWAR, H.A. (1975) The effect of tetranicotinoyl fructose (Bradilan) on fibrinolytic activity, platelet stickiness and some other parameters. *Journal of International Medical Research*, **3**, 423.

BOZADZHIEVA, E., KONSTANTINOV, N. & PENCHEVA, D. (1973) Experience in treating diabetic retinopathy with heparin. *Vutreshni Bolesti* **12**, 117.

CARROLL, W.W. & GEERAETS, W.J. (1970) Diabetic retinopathy and salicylates. *Annals of Ophthalmology*, **4**, 1019.

EGEBERG, O. (1963) Blood coagulability in diabetic patients. *Scandinavian Journal af Clinical and Laboratory Investigation*, **15**, 533.

FEARNLEY, G.R., CHAKRABARTI, R. & AVIS, P.R.D. (1963) Blood fibrinolytic activity in diabetes mellitus and its bearing on ischaemic heart disease in obesity. *British Medical Journal*, **1**, 921.

GIBELI, A., FRANDOLI, G. & GIAVOLA, P. (1966) Azione fibrinolitica del nicofuranosio in soggetti anzianii. *Gazzetta Internazionale di Medicina E Chirurgia*, **LXXI**, 3.

HAIM, S., SHAFRIR, A., HAIM, N. & LICHTIG, C. (1973) Microangiopathy in cases of granuloma annulare. *Dermatologica*, **147**, 261.

HANDELSMAN, M.B., MORRIONE, T.G. & GHITMAN, B. (1962) Skin vascular alterations in diabetes. *Archives of Internal Medicine*, **110**, 70.

HONERSKI, W. (1974) Durabolin in the treatment of diabetic proliferative angioretinopathy. *Klinika Oczna*, **44**, 461.

JOHNSON, F.L. (1975) Hepatoma associated with androgenic steroids. *Lancet*, **ii**, 1294.

KAPPERT, A. (1961) Experimental and clinical investigations with Hexanicit. *Therapeutische Umschauund medizinische Bibliographie*, **18**, 303.

MCMILLAN, D.E., BREITHAUPT, D.L., ROSENAU, W., LEE, J.C. & FORSHAN, P.H. (1966) Forearm skin capillaries in diabetics, potential diabetics and non-diabetic subjects; changes seen by light microscopy. *Diabetes N.Y.*, **15**, 251.

MINE, T. (1975) The progress and the fibrinolytic activity of diabetic retinopathy treated with dextran sulphate. *Acta Societatis Ophthalmologica Japan*, **79**, 371.

SOMMER, H. (1965) Nicotinic acid levels in the blood and fibrinolysis under the influence of the hexanicotinic acid ester of m-inositol (Hexopal). *Arzneimittel Firschung*, **15**, 1337.

Sodium, potassium and phosphorus content of normal and psoriatic skin

G.H.HAJINI, S.T.HUSSAIN *and* S.N.A.SHAH *Government Medical College, Srinagar, Kashmir, India*

It has recently been reported that the activity of membrane-bound Na^+/K^+ ATPase is reduced in the psoriatic lesion (Mahrle & Orfanos, 1974). Since there is evidence that a change in the intracellular concentrations of certain ions can trigger mitotic activity (Mazia, 1974), it seems possible that a disturbance of the Na^+ or K^+ levels could play a role in the pathogenesis of psoriasis.

We have attempted to test this hypothesis by the measurement of Na^+ and K^+ concentrations in normal and psoriatic skin; phosphorus was also assayed to provide an appropriate reference variable. Biopsies (5 mm diameter) were removed from psoriatic lesions, from the uninvolved skin of psoriatic patients and from healthy controls. After rinsing in distilled water, blotting on filter paper and

TABLE 1. Na$^+$, K$^+$ and phosphorus levels of normal and psoriatic skin

	Psoriatic skin	Uninvolved skin of psoriatics	Normal skin
Na$^+$ mmol/g wet tissue wt.	0·128 ± 0·003	0·132 ± 0·010	0·160 ± 0·024
K$^+$ mmol/g wet tissue wt.	0·050 ± 0·006*	0·030 ± 0·003	0·031 ± 0·004
K$^+$/Na$^+$ ratio	0·38 ± 0·005*	0·24 ± 0·024	0·24 ± 0·016
Total P mg/g wet tissue wt.	6·3 ± 0·50*	3·5 ± 0·23	3·3 ± 0·40
Total P mg/mmol Na$^+$	52 ± 10*	26 ± 4·3	26 ± 5·0

Values for total P refer to mean ± S.E. for 9 observations; all other values refer to mean ± S.E. for 10 observations.

* Significant at 5% level.

weighing, material was stored at −20° C prior to analysis. Na$^+$ and K$^+$ were estimated by flame photometry following wet ashing with nitric acid; phosphorus was assayed by the method of Fiske & Subbarow (1925) after ashing with magnesium nitrate and extraction of the residue with 5 N H_2SO_4.

Our results are shown in Table 1. The values for normal skin are in good agreement with those previously reported (Brown, 1926; Cornbleet *et al.*, 1947; Eisle & Eichelberger, 1945; Kandhari *et al.*, 1969). K$^+$ and phosphorus levels were significantly increased in the psoriatic lesion (both in absolute terms and also as a ratio to Na$^+$). No significant changes were found in the uninvolved skin.

The increases in K$^+$ and phosphorus in the lesion are wholly explicable in terms of its increased cellularity; indeed, the increased K$^+$/Na$^+$ ratio is in the opposite direction to any change which might result from a diminished activity of the Na$^+$/K$^+$ pump. Our findings therefore do not support the hypothesis of a disturbance of the electrolyte balance playing a causative role in the pathogenesis of psoriasis.

REFERENCES

BROWN, H. (1926) The mineral content of human, dog and rabbit skin. *Journal of Biological Chemistry*, **138**, 535.

CORNBLEET, T., INGRAHAM, R.C. & SCHORR, H.C. (1942) Calcium, potassium and sodium metabolism and the skin; the use of potassium chloride in certain allergic dermatoses. *Archives of Dermatology*, **46**, 833.

EISLE, C.W. & EICHELBERGER, L. (1945) Water, electrolyte and nitrogen content of human skin. *Proceedings of the Society for Experimental Biology and Medicine*, **58**, 97.

FISKE, G.H. & SUBBAROW, Y. (1925) The colorimetric determination of phosphorus. *Journal of Biological Chemistry*, **66**, 375.

KANDHARI, K.C., PASRICHA, J.S., DUGGAL, S.K. & PANDHI, R.K. (1969) Investigations into the causation of increased susceptibility of diabetics to cutaneous infections. *Indian Journal of Medical Research*, **57**, 1295.

MAZIA, D. (1974) The cell cycle. *Scientific American*, **230**, 54.

MAHRLE, G. & ORFANOS, C.E. (1974) Decreased membrane-bound ATP-hydrolytic activity in psoriatic epidermis. *British Journal of Dermatology*, **91**, 529.

First demonstration of cathepsin A in epidermis

G.VOLDEN *Hudavdelingen, Rikshospitalet, Oslo, Norway, and* JOSÉ J.M.A. VAN DEN HURK, P.D.MIER *and* H.ROELFZEMA *Department of Dermatology, University of Nijmegen, The Netherlands*

Cathepsin A (EC 3.4.2.-) is a carboxypeptidase of lysosomal origin with a specificity resembling that of pancreatic carboxypeptidase A. It is usually assayed by measuring the rate of release of tyrosine from the synthetic substrate N-carbobenzoxy-α-L-glutamyl-L-tyrosine (CBZ-glu-tyr) (Barrett, 1972). When applied to epidermis, however, the conventional methods of determining tyrosine, such as the ninhydrin reaction, do not provide sufficient sensitivity; in addition, the high levels of free amino acids in crude epidermal extracts give rise to unacceptably high blanks.

We have now succeeded in overcoming these difficulties by employing the new fluorescent amine reagent 'Fluram' (Roche) to determine free tyrosine after prior dialysis of the tissue extract. Guinea-pigs were killed by a blow to the head, shaved, and epidermis removed with a keratotome set to a depth of 0·1 mm. Epidermis (5 mg/ml) was homogenized in bovine serum albumin solution (1 mg/ml), centrifuged, and the extract dialysed twice against 0·15 M NaCl. Aliquots of 0·1 ml were incubated with 0·1 ml CBZ-glu-tyr (5 mM in 0·1 M acetate buffer, pH 5·0). After 1 h at 37° C the reaction was terminated by the addition of 1 ml borate buffer (0·2 M, pH 9·5), 0·5 ml of 'Fluram' (0·2 mg/ml) was added with rapid stirring, and the fluorescence measured ($\lambda_{EX} = 390$ nm, $\lambda_{EM} = 480$ nm). Appropriate blanks and tyrosine standards were included with each assay.

The average level of cathepsin A in guinea-pig epidermis was of the order of 1 μmol/min/g fresh weight, a much higher figure than that found for other cathepsins (Mier and v/d Hurk, 1975). The enzyme was rather stable, the time-curve being linear for at least 2 h at 37° C, and dialysed extracts showing no loss of activity during several days at 4° C. The pH curve was somewhat flat, optimum activity occurring in the range pH 4·5–6·0. The Lineweaver–Burk plot for CBZ-glu-tyr was linear in the range 0·2–5·0 mM, extrapolation yielding a K_m of about 0·5 mM. EDTA, Mg^{++}, Mn^{++} and F^- ions had no effect at 1 mM final concentration; cysteamine (1 mM) produced a small increase in activity (23%) and Ag^+ ions (1 mM) a slight inhibition (19%).

Since purified preparations of cathepsin A are reported to be unaffected by sulphydryl reagents (Barrett, 1972) these last results suggest that there may be slight interference from other exopeptidases present in epidermis. For the routine measurement of cathepsin A this difficulty could be overcome by the inclusion of Ag^+ in the incubation mixture.

REFERENCES

BARRETT, A.J. (1972) Lysosomal enzymes. In: *Lysosomes: a Laboratory Handbook* (Ed. by J.T.Dingle), p. 87. North-Holland Publishing Co., London and Amsterdam.

MIER, P.D. & VAN DEN HURK, J.J.M.A. (1975) Lysosomal hydrolases of the epidermis. 3. Peptide hydrolases. *British Journal of Dermatology*, **93**, 509.

British Journal of Dermatology (1976) **95**, 677.

Obituaries

DR G.B.DOWLING

Dr G.B.Dowling, Consulting Physician to the Department for Skin Diseases at St Thomas's Hospital and to St John's Hospital for Diseases of the Skin, died peacefully at St Thomas's Hospital on 1 June 1976 at the age of 84. He had long been an internationally revered authority on his speciality and the doyen of British dermatologists. He had been in failing health for several months, happily without lessening of his intellect, humour or courage. In the month before his death he attended with obvious

enjoyment the annual oration and dinner of the club which bears his name and also a dinner to mark the 250th anniversary of the founding of Guy's Hospital.

Geoffrey Barrow Dowling was born on the 9 August 1891 in Cape Town, where his father was first organist of the cathedral and a leading figure in the musical life of Cape Province. Educated at the Diocesan College in Cape Town, at Exeter Cathedral Choir School, Dulwich College and Guy's Hospital, his medical training was interrupted by 2 years service as a cavalry trooper in the First World War. In 1920 he graduated MB, BS with honours in medicine and in the same year became MRCP London. He proceeded to MD in 1921 and was elected FRCP in 1933. At Guy's he held several house officer and registrar appointments in medicine and pathology and his outstanding ability was soon recognized. His decision to specialize in dermatology owed much to the influence of Dr H.W.Barber. In 1926 Dowling was appointed Physician to St John's Hospital for Diseases of the Skin, a post to which later was added the charge of the Goldie Leigh Hospital for the treatment of skin diseases in children, including fungus scalp infections. In 1927 he was appointed Assistant Physician to the Skin Department at Guy's. He was also in charge of the Skin Department of the West London Hospital, resigning these two latter posts on his appointment to St Thomas's Hospital in 1933. In addition he was Civilian Consultant in Dermatology to the Royal Air Force.

Dowling was largely responsible for the renaissance of dermatology in this country during the past 25 years and many would hold that his influence has been unequalled in the past century. His commanding intellect and classical humility, enhanced by unyielding and selfless zeal, made him the outstanding figure in his speciality from the late 1920's onwards. He was a superb clinician with an exceptional talent for distinguishing between the significant and the irrelevant. He realized the importance of exact observation and description and in this respect perhaps the French school ranked highest in his esteem. To an even greater degree his influence was manifest in the clarity of his thinking, whether in individual diagnosis or in investigative studies, which were at that time predominantly clinical. With his formidable and occasionally devastating critical faculty he drew a sharp distinction between speculative theory and proven fact, whilst appreciating that some well tested empirical observations would have to await a rational explanation. With his keen and enquiring mind he perceived that in many fields further advances would have to rely on experimental investigation, which at that time was in its infancy and limited to a few brilliant workers such as Haxthausen, for whom he had a lasting respect and affection. His early experience in pathology led him to encourage the study of the histopathology of the skin. This encouragement was within a few years to ensure its pre-eminence in this country. When in 1952 Dowling was appointed the first Director of Medical Studies at the Institute of Dermatology, he greatly encouraged investigative studies, particularly in photobiology and in contact sensitivity, the results of which were to achieve world wide fame.

His original contributions were outstanding. They began with the investigations which he carried out with Dr J.H.H.Macleod on the role of *pityroporon ovale* in seborrhoeic dermatitis. Later in conjunction with Dr Freudenthal he became interested in the relationship between dermatomyositis and scleroderma. This culminated in his authoritative Watson-Smith lecture to the Royal College of Physicians in 1953. His greatest contribution was the demonstration in 1945 of the efficacy of massive doses of calciferol in the treatment of lupus vulgaris. This was no chance discovery but the outcome of simple and logical deduction from the knowledge that the only previous significant treatment had been concentrated ultraviolet rays, a technique devised by Finsen, very time consuming and available to few. The same therapeutic advance was introduced independently and simultaneously by Charpy in France, but neither knew of the other's work until after the war. Although calciferol was soon superceded by other anti-tuberculous drugs, Dowling's treatment gave the first hope for the majority of sufferers from this age old and often grossly disfiguring disease, which at that time was still not uncommon. His colleagues deeply regretted that he did not receive the public honour which he so justly merited.

His retirement from his hospital appointments in 1956 signalled the beginning of a second period of unrelenting professional activity, during which he became an almost legendary figure. For many years he gave invaluable service as a locum consultant, with very busy clinics at Lewisham General Hospital and at Farnham Hospital in Surrey. For him the enjoyment of the work more than compensated for the difficulties of public transport, often having to leave his house before dawn. Even in his late seventies he retained an undiminished interest in dermatology, continuing to make valuable and often original contributions.

His pre-eminence owed much to his original work but even more to his immense and lasting influence on his juniors. By the mid 1950's over half the dermatological consultants appointed in the United Kingdom during the previous decade had been trained by him. Many of the papers written by his registrars, some attaining international recognition, derived from Dowling's suggestions and help. With characteristic modesty and his earnest wish not to detract from his junior's success he invariably declined to allow his name to appear as co-author. His reputation also attracted first class postgraduates from overseas, particularly from Australia and South Africa. It was also characteristic of Dowling that he did not restrict his help to his own registrars. He initiated the regular Saturday morning meetings at St John's Hospital for Diseases of the Skin, open to all junior dermatologists and which now enjoy an international reputation. His success with postgraduates of all ages and from many countries began with his kindness, enthusiasm and his obvious pleasure in their company. Dowling taught them by example rather than by didactic precept but they soon came to appreciate the clarity of his thinking and his immense knowledge. His interest in their welfare did not cease when they became consultants and to all he became a dearly loved friend.

Much of the immense influence that he was to have on dermatology in this country and overseas began in 1948, when he started an informal journal and travelling club which was later to bear his name. The object of the club has always been to promote knowledge and friendship among younger dermatologists and it now has a membership of over 300, including members from most European countries, the Middle East, Africa, North and South America. Regular and highly successful meetings have been held in most of the regional centres in the United Kingdom. The distinction and kindly charm of the founder has enabled equally successful visits to be made to most of the main centres in Europe and also to Iran, Israel, Lebanon and South Africa. To commemorate the great service rendered by its founder the club contributed generously to a fund to establish an annual Dowling oration. The British Association of Dermatology also paid him the unique tribute of founding a Dowling Fellowship for young dermatologists to undertake research projects for upwards of a year. Whilst characteristically disclaiming any personal credit for the help that he gave to young dermatologists, the long and increasing success of the club, reinforced by the Dowling Fellowship, probably gave him the greatest single pleasure of all his achievements and distinctions.

In his earlier years it was not always easy for the newcomer to detect the qualities which made him so pre-eminent and beloved. His kindly and scholarly bearing were obvious to all, but modesty and intense concentration sometimes made him appear diffident and preoccupied, an appearance enhanced in the clinic by a magnifying visor which temporarily rendered him incommunicado. His abhorrence of appearing to patronize often led him to overestimate the erudition of his audience, and with a less than perfect enunciation his observations were not always clear to the uninitiated. For these reasons he was perhaps less than successful as a teacher of undergraduates, to whom he nonetheless endeared himself by his great kindness and occasional mannerisms. It may come as a surprise to the younger dermatologists of today to be told that in middle life Dowling appeared older than his contemporaries and to generations of St Thomas's he was affectionately known as 'Daddy Dowling'. With a great appreciation of music, his interest in the student's welfare found a happy expression when he became President of the St Thomas's Hospital Musical Society, which was actively sponsored for many years by Dr Ralph Vaughan Williams who dedicated a composition to the Society.

Dowling preferred hospital to private practice but to all his patients he gave unflagging devotion. His kindness and attention to their problems commanded their affection and respect, although full understanding of his advice often had to await the arrival of a letter to their general practitioner. A man of strong character and prodigious stamina, his rational outlook was in keeping with a stoic but cheerful philosophy, enabling him to recover rapidly from adversity. This same philosophy masked great compassion and occasionally deep emotion. His humility was deeply rooted but he often found it difficult to accept any deviation from his own high standard of moral and intellectual integrity. The tolerance that he showed to his juniors did not always extend to his contemporaries and occasionally his judgments were less than fair. Further acquaintance often modified an adverse judgment and led to a warm personal friendship. Criticism was not invariably welcome but if genuine provoked only fleeting displeasure. He had a very keen sense of humour but on occasion he was known to mistake the friendly badinage of a colleague for an attack on dermatology itself. His loyalty to his colleagues was absolute and no junior ever had a kinder chief. Any trace of autocracy and pretension were anathema. The occasion when a new nurse mistook him for the barber caused him as much amusement as it embarrassed the present writer, then his house physician. The friendly title of Headmaster which he soon acquired after the founding of the Dowling Club initially gave him some misgivings until he realised it was a token of affection. His love of freedom led him to distrust any idea which smacked of organisation. In committees, which he greatly disliked and avoided if possible, he was not very successful, and he had no taste for intrigue. In an age when bureaucratic planning is in the ascendant it is salutary to recall that Dowling's world wide influence began almost spontaneously and has continued to flourish without any trace of rigid organisation. He felt deeply about men and affairs. Injustice he sought to rectify whenever possible, preferring reasoned argument to angry words which were quite foreign to his temperament. His opinions were often forceful but devoid of any trace of malice. Not infrequently they found expression in his characteristically audible 'asides' at medical meetings.

Dowling was a man of wide culture with an extensive knowledge and appreciation of literature, music and painting; his interest in the latter being greatly stimulated by the accomplishments of his family. He was very interested and knowledgeable in many branches of sport. In his youth he was no mean rugby player and later became a competent golfer. The motorcar held little attraction for him except as a means of transport. His style of driving was unorthodox and perhaps on occasion perilous but accidents were few and trivial. In his seventies he became an enthusiatic and proficient cook, a hobby which afforded him much pleasure.

Socially, his gentle and benevolent charm at once endeared him to the newcomer and to his friends of all ages and all nationalities. His company was always a delight. The success of any gathering large and small was assured by his stimulating conversation, a keen sense of humour and a dry wit. His gregarious habits and sense of fun astonished the stranger as much as they delighted his friends. The prodigious stamina that was such a feature in his professional life extended also to his social activities, as many of his juniors could testify. All who have had the good fortune to have known him cherish many happy, affectionate and unforgettable memories of his friendship.

Many professional honours came to him. He was President of the British Association of Dermatology and was later made an Honorary Member. He was President of the Dermatological Section of the Royal Society of Medicine and was later elected Honorary Fellow of the Society. All the major dermatological societies of the world conferred on him their Honorary Fellowships. He was President of the St John's Dermatological Society and gave the Prosser White Oration in 1958. An Honorary Doctorate of Medicine was conferred on him by the University of Utrecht in 1957 and in 1959 he received an Honorary Doctorate of Medicine from the University of Pretoria. In 1972 he was awarded the Alfred Marchionini Gold Medal. He married in 1923 Mary Elizabeth Kelly, who died in 1965. They had two sons and two daughters. To them we send our warmest sympathy in affectionate memory of a very great man.

HUGH WALLACE

JUDITH SAVATARD

Dr Judith Savatard, formerly Consultant Dermatologist to the Manchester and Salford Hospital for Skin Diseases, died on 25 August 1976. She was a graduate of the Royal Free Hospital in the early twenties and married Dr Louis Savatard, the internationally renowned histopathologist, in 1925. From about 1940 she attended as Consultant Dermatologist and Venereologist, the Manchester Royal Infirmary, the Duchess of York Hospital for Babies and the Manchester and Salford Hospital for Skin Diseases, until her retirement some ten years ago, and during these years her qualities of good sense, thorough competence, modesty, and loyalty to traditional medical practice and ethics were greatly appreciated by her colleagues.

She is survived by her son a doctor, and two daughters, one of whom is also medically qualified.

J. CURRY

DR W.B. REED DR M.FARB AND DR W.W.GUNTHER

Dr W.B.Reed of California died on 15 June 1976. He had been a honorary foreign member of the British Association of Dermatologists since 1973.

Dr M. Farb died in late 1975, and Dr W.W.Gunther in July 1976. Both were members of the New South Wales Branch of the British Association of Dermatologists. Their obituaries will appear in full elsewhere.

British Journal of Dermatology (1976) **95**, 682.

Correspondence

SIR, We would like to point out that there is a regrettable lack of clarity in Figs 3 and 4 of Dr D.Munro's paper in your supplement on the Glaxo Symposium (BJD, Supplement **12**, Vol **94**, March, 1976, pp. 70–71).

These graphs have different ordinates and abscissae which make visual comparison impossible. The uninitiated reader might well miss the point that the application under polythene occlusion of 0·1% betamethasone 17-valerate ointment causes significant and sustained reduction in the serum cortisol, whereas 60 g of hydrocortisone 17-butyrate cream does not.

Nor was it apparent from the diagram that the quantity of hydrocortisone 17-butyrate cream used was twice the amount of betamethasone 17-valerate ointment. One had to search the text to find that piece of information.

Department of Dermatology,
Skin Hospital,
Manchester
P.J.AUGUST

Medical Adviser,
Brocades (GB) Ltd.
I.I.DAINOW

Book Reviews

Progress in Allergy. Edited by P.KALLÓS, B.H.WAKSMAN, and A.DE WECK (1976) Vol. 20. S. Karger, Basel, Switzerland. Price US $ 49.25.

This volume is an exhaustive reappraisal of recent work on the thymus, lymphocytes, macrophages and polymorphonuclear leukocytes and the parts these play in immunity and tissue injury. It is meticulously written and divided into four sections, each of which ends with copious references.

In the first section, Cantor and Weissman describe the part played by the thymus in the development of the T. lymphocyte and the function of this cell in immune responses. In section two, Oppenheim and Rosenstreich, in a beautifully informative chapter, discuss the action of mitogens, antigens and allogeneic cells on lymphocytes and the mechanisms whereby these activators work. The role of the macrophage in the binding, processing and presentation of stimulants to the lymphocytes and the effect of serum factors are fully discussed and explained. In section three, Wedner and Parker describe lymphocyte metabolism in all its aspects with particular reference to lymphocyte activating agents and the biochemical changes which occur in these cells after activation. In the final section Goldstein presents all the recent work on polymorphonuclear leukocytes and their lysosomal enzymes, giving a full and clear account of the mechanisms of their release and the part these enzymes play in tissue injury and blood fibrinolysis.

If there is a criticism of this book it is that there is no index, although each section is prefaced by a full list of contents. It contains a mass of clearly presented and concisely written information and is a necessity for both immunologists and those with an interest in immunology. On reading it one is left with a feeling of intense admiration for the authors who have presented so clearly such a volume of information in one small book.

E.L.RHODES

Dermatological Photobiology: Clinical and Experimental Aspects. I.A.MAGNUS (1976) Oxford: Blackwell Scientific Publications. Pp. 292, illustrated. Price £9.75.

Clinical photobiology has developed in recent times because of the availability of equipment which allows for irradiation of the skin with selected wavelengths of UVR and light, the purity of which can be ensured and the energy accurately measured. The first eight chapters of this book are concerned with an introduction to the basic physics of electromagnetic irradiation, dealing with the physical and chemical effects of this irradiation, with particular reference to the skin, and in discussing sunlight and the factors which affect the solar spectrum. Then follows a detailed but easily understood description and assessment of the available artificial sources for irradiation of the skin, along with a discussion of the means available for measuring the emitted wavelengths, both from the point of view of purity of emission and of energy output, ending with a description of irradiation monochromators. This is followed by an assessment of the techniques used in the study of skin photosensitivity in the human subjects, in the experimental animal, and in the laboratory, stress being laid on the importance of accurate objective observation and of the appreciation of the normal as well as the abnormal response. Although the clinician may turn with increasing interest to the second half of the book—that is chapters 12 to 18—it is in these first 160 pages that lies the knowledge required by any dermatologist who intends to develop a capability for investigating the clinical problem of skin 'photosensitivity'.

Dermatologists have reached the stage in the understanding of photosensitivity reactions of the skin where it is now possible to list entities such as polymorphic light eruption, solar urticaria, summer prurigo, hydroa vacciniforme, the chronic photosensitivity dermatitis and actinic reticuloid syndrome, and photocontact dermatitis, and to define them clearly based on clinical and photobiological features. In addition, the use of action spectrum studies has helped to determine whether photosensitivity exists or not in a long list of dermatoses which include atopic eczema, psoriasis, lupus erythematosus, etc., etc. Although this understanding has not necessarily led to the discovery of specific causal factors, it has been shown to be essential to the care and treatment of photosensitive subjects. This is explained and illustrated by the author in chapters 12–14 dealing with the photodermatoses which contain much of the author's unique experience over the last 20 years or so. This is followed by a discussion of the drug and chemical-induced photosensitivity reactions and the relevance of phototoxicity and photo-allergy and the state of persistent light reaction, and goes on to the porphyrias where the author's original contribution is acknowledged by all. Lastly, we have a chapter on treatment.

The author makes a point early in his book of assuring the reader that his views do not necessarily coincide with those held by all. It is Professor Magnus' strength as an investigator, and an observer, that he manages to maintain an objective approach which does not often allow for the weaknesses inherent in the wish of some to see 'photoallergy' where none may exist; or to seek after the photoactive substance amongst the idiopathic photodermatoses. He may however have to continue to bear with those who have visions and dream dreams. It is the privilege of a reviewer to say such things and perhaps live to regret them. Also, not all would agree with some of the views expressed on photosensitivity, eczema, summer prurigo, and the specificity of the entity of actinic reticuloid. It will be interesting to see in the second edition of this invaluable book whether photoactive substances, be they acting in a phototoxic or a photoallergic way, have become 'decent' and accepted or not. By then phototherapy, photochemotherapy, and 'photomedicine' should be with us to stay. It must be very satisfying to have written such a book which is going to last for a long time and which will have the advantage of future editions which will describe the inevitable progress of medical photobiology.

All those who read this book, and they will be many, will have gained access to a branch of medicine which the author has the experience to assess and to describe. Professor Magnus holds a unique position in the dermatological world and therefore this monograph on The Clinical and Experimental Aspects of Dermatological Photobiology will rightly become for the clinician the standard work in the future.

W.FRAIN-BELL

The Management of the Menopause and Post-Menopausal Years. Edited by Stuart Campbell (1976) Lancaster: Medical & Technical Publishing. Pp. 440.

This volume contains the Proceedings of the international symposium held in London in November 1975. The speakers were drawn from many medical and surgical specialities and from several countries, and they included also general practitioners and journalists. One of the latter, Wendy Cooper, gave the opening paper and it was of course appropriate that she should have been invited to do so since her writings, notably her book, '*No Change, a Biological Revolution for Women*', have given HRT (hormone replacement therapy) wide publicity, and have forced many doctors to enquire more closely into the present state of our knowledge of the menopause in order to answer their patients' questions and assess both the alleged benefits and the possible hazards of long-term oestrogen therapy.

This symposium was planned to bring together recognized authorities engaged on clinical or laboratory research on the psychological and physical effects of the loss of ovarian function, on the effects, desirable or undesirable, of exogenous 'natural' or 'synthetic' oestrogens, and on the management of the post-menopausal woman with or without symptoms. The 39 papers, and the fully reported discussion which followed each session, make fascinating reading for all physicians; they provide well informed reviews, but they also reveal surprisingly large gaps in our knowledge. Mr R.J.Beard, for example, stated that the only 'menopausal symptoms' consistently relieved by oestrogens are flushing, sweating, and dryness of the vagina, and most clinicians would agree with him. But Professor Campbell found that the correlation between falling or low levels of plasma oestrogen or androstenedione and flushes was not close enough to prove a causal relationship. The relationship is likely to prove infinitely more complex as other excellent investigations on the endocrine status of menopausal women are showing.

Of more specialist interest to the dermatologist are two papers on the effects of oestrogens on the skin. Marks and Shahrad of Cardiff gave a competent review of the literature which is surprisingly scanty and often conflicting; they also presented a preliminary report of their studies on the effects of oestrogens on dermal thickness. Rauramo of Finland described his research on the effects on the epidermis of castration and of subsequent oestrogen administration.

The editor in his foreword expresses the hope that specialist readers will not confine themselves to the papers directly related to their own field. Certainly dermatologists should avoid doing so, for with few exceptions all the papers in this book are relevant to the practising clinician who is interested in disease mechanisms. The contraindications to the administration of oestrogens should be as widely publicised as their undoubted virtues. The urgent need for further research is evident, and to this research dermatologists could make an important contribution.

Arthur Rook

The Acnes, Clinical Features, Pathogenesis and Treatment. W.J.Cunliffe and J.A.Cotteril. Volume 6 in series. *Major Problems in Dermatology* (1975) London: W.B. Saunders. Pp. 306. Price £8.50.

The Acnes' is a worthy addition to the highly successful series on Major Problems in Dermatology. This volume is written by two of the young disciples of the Newcastle school, who are well qualified to chronicle and comment on the massive amount of research which has been done in the last decade.

Most of our recent understanding of acne has stemmed from studies of sebum excretion, which was made possible by the reproducible method of sebum collection described by Strauss and Pochi in 1961. A large part of the book is devoted to the adaptation and application of this method which has yielded so much. This has been widely used to elucidate further the physiology of the sebaceous gland and also monitor objectively the effects of therapeutic agents.

It seems now that this well of sebum may be beginning to run dry, and the authors point the way to other approaches. Although we have some clues about endocrine factors which may produce acne we still have little idea what switches it off, for even the seborrhoea continues after the acne years. General endocrine studies and a search for a sebotrophic hormone are as yet inconclusive, and it does seem that the vital clues may be buried locally in the pilosebaceous units, which possibly have the ability to detoxicate androgens or metabolise precursors to more potent androgenic hormones. It could be that it is even more subtle than this and that receptor sites at a cellular and sub-cellular level are involved.

The book is balanced by an interesting review of the clinical types of acne and a critical evaluation of the therapeutic measures which have brought considerable relief to the acne sufferer. Most of the therapeutic agents in current use are reviewed and doubts are rightly cast on the effects of sulphur which may be comedogenic. I, personally, was also impressed by the authors' sensible comment that a sympathetic explanation must be given to the patient of our approach to their problem, and that patience is required both from the sufferer and doctor in tackling this distressing complaint which can now be greatly alleviated.

In conclusion, I would say this book should be on the shelves of all Departmental Libraries, because it is of interest to those who are developing an interest in the investigative aspects of this complex disease, it provides a comprehensive review for those involved in investigative work and it also has much for the practising dermatologist.

G.W.BEVERIDGE

Self Assessment of Current Knowledge in Allergy. Edited by CLAUDE ALBEE FRAZIER (1976) Flushing, N.Y.: Medical Examination Publishing Company. Pp. 169. Price US $10.00.

This small, loose-leaf, book contains on its 169 pages 860 multiple choice questions covering subjects ranging from urticaria to drug allergy, insect sting allergy and 'immuno-dermatitis'. The matching number of answers are fortunately supported by references, which, however, unfortunately are not all up-to-date. Hence not all answers are correct (for instance platinum salts may cause asthma).

The editor has been supported by 18 experts in the fields of allergy and clinical immunology. This book is likely to be useful to all who have to undergo trial by multiple choice papers, either by having to identify the correct answers or by having to set questions which do not discriminate against the more knowledgeable candidate. It will also be of interest to those who seek light entertainment in this somewhat controversial corner of medicine and to those who wish to tease their friends or enemies with the help of such a pocket-size source of expert information—as long as they realize that not all answers are correct!

G.HOLTI

British Journal of Dermatology (1976) 95, 686.

News and Notices

XV INTERNATIONAL CONGRESS OF DERMATOLOGY.
16–22 OCTOBER 1977, MEXICO CITY

SCIENTIFIC PROGRAMME

The Scientific Programme will include:

5 Special lectures; 25 Topics of current interest; 15 Symposia; 11 Courses; 40 Workshops; 75 Case presentations; 15 Free communication sessions; 5 Poster communication sessions; 10 Film projection sessions; 50 Informal discussion groups.

The official languages of the Congress are Spanish, English, French, German.

Simultaneous translation services in the official languages of the Congress will be provided during the main scientific sessions.

Themes of special lectures

Mediators of immunological reactions; Delayed hypersensitivity; RNA—viruses and tumorigenesis; Progress in the biology of macrophages; Biosynthesis of melanin.

Themes of topics of current interest

Diagnosis and management of ichthyotic conditions; Histocompatibility in skin diseases; Immunotherapy; New eczematogenes; Prostaglandins; The pre-lymphomas; Dermatological aspects of hyperlipoproteinemias; Antiandrogens in dermatology; IgE & Rast test in dermatology; Immune complexes in skin diseases; Immunology of malignant melanoma; Clinical cutaneous microbiology; Cutaneous paraneoplasias; Diagnosis and classification of physical urticarias; Hair loss; Nodular dermatoses of the legs; Skin reactions due to food and additives; Selective immunosuppressive therapy; New occupational dermatoses; Nonsteroid anti-inflamatory agents; Candidiasis; Pathophysiology of the nail; Epidermal necrolysis; Advances in acne treatment; Advances in the management of psoriasis.

Themes of symposia

Recent advances in: Psoriasis; Vasculitis; Dermatological mycology; Syphilis and venereal diseases; Bullous diseases; Dermatological oncology; Pigment disorders; Drug eruptions; Virus diseases of the skin; Contact dermatitis; Genodermatoses; Acne; Leprosy; Photodermatoses; Porphyria.

Themes of courses

Immunology; Pathology; Photobiology; Structure and function of the skin; Dermis and connective tissue; Physical methods in dermatology; Tropical dermatology; Diseases of the oral mucous membranes; Dermatological surgery; Paediatric dermatology; Cosmetic dermatology.

Themes of workshops

Chemistry and structure of keratinization; Epidermal regeneration; Epidermal cellular kinetics; Dermal–epidermal interaction; Collagen; Hair growth; Percutaneous absorption; Methods in cutaneous pharmacology and drug testing; Tissue culture of isolated systems; Macrophages; Chemical mediators of inflammation; Lymphocytes and lymphokines; Complement; IgE in dermatology; Diseases due to immune complex formation; Immunofluorescent techniques as diagnostic aids; Immunologic aspects of pemphigus and pemphigoid; Immunology of infertility; Immunology of melanoma; Oncogenic viruses of the skin; Mechanisms in atopic dermatitis; Mechanisms in urticaria; Mechanisms in contact dermatitis; Mechanisms in photosensitivity; Genodermatoses; Mechanisms in acne; Microbiology; Melanin; Electron microscopy and freeze fracture techniques as an experimental tool; Scanning electronmicroscopy; Cyclic AMP & cyclic GMP; Mechanisms in psoriasis; Sweating; Utraviolet & chemical carcinogenesis; Hormonal control of skin appendages; Mechanisms in dermatitis herpetiformis; Blood supply of the skin; Structure and function of cutaneous innervation.

Congress enrolment

Fees

	Until 15 April 1977	After 15 April 1977
Participants	US $140.00	US $160.00
Accompanying persons	US $50.00	US $65.00

Payment of the enrolment fee entitles participants to attend the scientific sessions and social activities, and to receive a copy of the Congress Programme and Book of Abstracts. All registered participants may present case presentations, free communications, scientific exhibits, and films. They may also participate in the Courses and Workshops by completing and returning the corresponding application forms. Registered accompanying persons may participate in all social events including those especially organized for the ladies.

Early enrolment
Persons wishing to attend should complete the enrolment form and return it together with a bank draft, before 31 July 1977, to the General Secretariat, XV Congreso Internacional de Dermatologia, Avenida Veracruz 93, México 11, D. F. Mexico.
Bank draft should be made to the order of: XV Congreso Internacional de Dermatologia.
The General Secretariat will mail a receipt, exchangeable for the Congress documents at the Congress Building beginning Friday, 14 October 1977, 9.00 a.m. to 5.00 p.m.

Last-minute enrolment
Those who have been unable to do so before, may enrol at the Congress Building beginning 14 October 1977, from 9.00 a.m. to 5.00 p.m.

Cancellations
Fees paid will be refunded minus 10% on cancellations received by the General Secretariat before 31 August 1977. Fees are not refundable if cancellation notice arrives after that date.

Publications
The official programme of the Congress will be included with the documents delivered to participants at the time they register at the Congress Building.
The book of abstracts will also be included with the participants' documents upon registering.
The proceedings will contain texts in extenso of Special Lectures, Topics of Current Interest, Symposia, Notes of Courses, and Summaries of Workships and Case Presentations. This volume will be published in English and may be ordered from the publisher's representative at the Congress Building.

Exhibitions
Exhibits will be installed in the exhibition area of the Congress Building.

Technical exhibition 'Derma-Expo 77'
Manufacturers and distributors will show the latest technical developments in medical products and equipment in an area of 1500 m^2.

Cosmetology exhibit
Recent advances in this area of dermatology will be on view.

Scientific exhibit

One section of the exhibition area will be devoted to graphic representations of research projects and the latest technical advances in dermatology.

Applications to participate, with a description of the material not exceeding 100 words, must arrive at the Congress General Secretariat by 31 January 1977.

The Organizing Committee will provide adequate space for the displays at no cost to exhibitors. Expenses involved in transportation, mounting and dismantling of the material and any modifications or supplementary installations, as well as charges originating in customs handling, will be at the expense of the exhibitors.

Applicants will be notified in due course if their exhibits have been accepted, as well as the place assigned them in the exhibition.

Art exhibit

The Organizing Committee will present an interesting collection of pre-Columbian sculptures depicting different types of skin disorders.

British Journal of Dermatology (1976) 95, 689.

News and Notices

Royal Colleges of Physicians

The following Dermatologists have recently been elected to the F.R.C.P. of

Edinburgh	Dr J.A.McConnachie
	Dr G.Palmer
	Dr H.K.El-Shamy
	Dr W.Susskind
	Dr P.Yesudian
Glasgow	Dr L.Stankler
Ireland	Dr Grace Allen

APPOINTMENT

University of Dundee

Dr Brian Johnson has been appointed Senior Lecturer in Photobiology in the University of Dundee. He obtained his BSc in physiology and zoology in London University in 1958. Thereafter he worked in the Department of Photobiology at the Institute of Dermatology in London, writing his PhD in 1965 on 'Physiological Effects of UVR on Mammalian Skin'. Subsequently he worked in the New York Hospital-Cornell Medical Center where he became Assistant Professor in the Division of Dermatology, until he was appointed Lecturer in the Department of Dermatology in Dundee in 1970.

Dr Johnson is well known for his publications in the field of skin photobiology, and, in particular, for his studies of the role played by lysosomes in photobiological processes.

Course announcement

The Seventh Annual Conference on the Immunopathology of the Skin organized by Dr Ernst H. Beutner and his associates at the State University of New York at Buffalo, by Drs Stefania Jablonska and Tadeusz P. Chorzelski of Warsaw, Poland and by Dr. Beno Michel of Case Western Reserve University in Cleveland will be given on May 26–28, 1977 by the Departments of Microbiology and Dermatology through Continuing Medical Education of SUNY/B at the Sheraton East in Buffalo, New York immediately following the Second Annual Westwood Conference organized by Dr Richard L. Dobson and his associates.

The first part of this conference on 26 and 27 May on 'Immunopathology of the Skin for Practising Clinicians' is designed primarily to afford practitioners a basic understanding of recent advances in the field and to enable them to utilize present knowledge of this subject in their practice. Registration fee is $95.00. For further information on registration write to Mrs Gloria Griffin, 219 Sherman Hall, SUNY/B, Buffalo, New York 14214, U.S.A.

The second part of this conference which is scheduled for 28 May 1977 will be a workshop for laboratory workers interested in presenting their own findings on the immunopathology of the skin and related problems. Titles for this workshop should be submitted before 31 January 1977 to Dr Ernst H. Beutner at the Department of Microbiology, Sherman Hall, SUNY/B, Buffalo, New York 14214, U.S.A.

Preliminary announcement

The second course in Investigative Techniques in Dermatology will be held at the University Hospital of Wales, Heath Park, Cardiff on Wednesday, Thursday and Friday the 14, 15 and 16 September, 1977. Further details will be announced shortly. Please address enquiries to: Dr R. Marks, Senior Lecturer and Consultant, Department of Medicine, Welsh National School of Medicine, Heath Park, Cardiff.

Index

SUBJECTS

Acanthosis nigricans; unilateral epidermal naevus resembling, 433
Acne vulgaris:
  effect of 1% cyproterone acetate in Cetomacrogol cream BPC (Formula A) on sebum excretion rate in patients with, 427
  effect of long term tetracycline treatment for on occurrence of R factors in the intestinal flora of man, 311
  laboratory and clinical study with special reference to microbial factors in pathogenesis (thesis summary), 571
  observations on the number and size of pilosebaceous ducts in, 153
  role of bacteria in (thesis summary), 453
Acnitis (case report), Suppl. 14, 58
Acropigmentation, reticulate, of Kitamura, 437
Actinic damage: possible inducer of post-inflammatory elastolysis and cutis laxa (letter), 105
Albumin, intravascular, and its transcapillary escape rate, and plasma volume in patients with extensive skin disease, 519
Alopecia areata, urticaria and dermatitis herpetiformis; complement component profiles in, 145
  congenital triangular, 657
  totalis and diffuse alopecia; auto-antibody profile in, 61
Anaemia, hypoplastic; associated with pyoderma gangrenosum and tabes dorsalis (case report), Suppl. 14, 69
Anthralin (dithranol) versus triacetoxyanthracene: investigations *in vitro*, 193
Antibiotics:
  antibiotic/corticoid combination in the treatment of impetiginized atopic dermatitis; co-operative double-blind trial, 323
  clomocycline in the treatment of persistent palmoplantar pustulosis; double-blind trial, 317
  epicillin capsules in treatment of skin infections, 177
  production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16
  rifampicin, inducing pemphigus, 445
  tetracycline treatment, long term, for acne vulgaris; effect of on occurrence of R factors in the intestinal flora of man, 311
Antifungal agent: Ciclopirox in treatment of superficial dermatomycoses, 83
Antigens:
  HL-A in guttate psoriasis, 163
  HL-A in lichen planus, 169
  HL-A in vitiligo, 173
Arthus-like responses to bacterial antigens without specific antibody in eczema, 379
Aspirin, benzoates and azo dyes; follow-up study of patients with recurrent urticaria and hypersensitivity to, 19
Atrophic blanche lesions closely resembling malignant atrophic papulosis (Degos' disease) in systemic lupus erythematosus, 649
Atrophy, dermal, induced by topical steroids; measurement of using a radiographic technique (summary), Suppl. 14, 12
Auto-antibody profile in alopecia totalis and diffuse alopecia, 61
Autoradiography, in the study of the epidermis in thyroid disease, 513

Bacteria:
  bacterial flora in psoriasis, 603
  hypersensitivity to, in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci, 285. III. Arthus-like responses to bacterial antigens without specific antibody, 379. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens, 493
  of human skin: effects of occlusion upon population dynamics of, 607
  of human skin: *in vitro* studies of antagonism among (thesis summary), 571
  role of in acne vulgaris (thesis summary), 453
Behçet's syndrome, familial, 637
  histological and direct immunofluorescence study of cutaneous hyperreactivity in, 631
Benzoates, aspirin and azo dyes; follow-up study of patients with recurrent urticaria and hypersensitivity to, 19
Biochemistry:
  changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats, 255
  differentiation of dermatophytes by biochemical and serological methods (thesis summary), 453
  effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins, 397
  kinetics of *in vitro* bovine keratohyalin synthesis, 115
  leukocyte chemotaxis in psoriasis scale, 1
  lysosomal hydrolases of the epidermis: 6. Changes in disease, 271

- Biochemistry (*cont.*)
  - lysosomal storage disorder of the epidermis characterized by deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627
  - physical training and connective tissues in young mice: biochemistry of skin, 525
- Bisalbuminaemia (case report), Suppl. 14, 54
- Black light and 8-methoxypsoralen therapy; effects and side-effects of (summary), Suppl. 14, 21
- Blaschko, lines of, 349
- Book reviews:
  - *Allergic Drug Reactions*, H.E.Amos, 572
  - *Applied Medical Microbiology*, J.G.Collee, 574
  - *Cancer in Children. Clinical Management*, ed. H.J.G. Bloom, J.Lemerle, M.K.Neidhardt and P.A.Voute, 334
  - *Clinics in Rheumatic Diseases*, Vol. I, no. 3. *Systemic Lupus Erythematosus*, ed. Naomi F.Rothfield, 107
  - *Control of Hospital Infection: A Practical Handbook*, E.J.L.Lowbury, G.A.J.Ayliffe, A.M.Geddes and J.D.Williams, 107
  - *Current Dermatologic Management*, ed. S. Maddin, 333
  - *Dermatological Photobiology: Clinical and Experimental Aspects*, I.A.Magnus, 683
  - *Dermatologie*, W.Braun, 217
  - *Dermatology*, S.L.Moschella, D.M.Pillsbury and H.J.Hurley, 454
  - *Dermatology*, H.Rorsman, 454
  - *Dermatophilus Infection in Animals and Man*, ed. D.H.Lloyd and K.C.Sellers, 334
  - *Diagnostic Pratique des Maladies des Peaux Noires*, A.A.de Terwangne, 573
  - *Drugs and Disease*. Symposium organized by the Royal College of Pathologists delivered in London, February 1975, ed. Sheila Worlledge, 108
  - *Handbuch der Haut- und Geschlechtskrankheiten*, J.Jadassohn. Ergänzungswerk. III/3A. *Nicht Entzündliche Dermatosen III. Gut- und Bösartige Neubildungen der Haut. Sklerosen.* Ed. H.A.Gottron and G.W. Korting, 332
  - *Histological Typing of Skin Tumours*, ed. Seldam and Helwig, 333
  - *Morphogenesis of Skin*, P.Sengel, 215
  - *Oral Mucosa in Health and Disease*, ed. A.E.Dolby 108
  - *Patch Testing*, S.Fregert and H.J.Bandmann, 215
  - *Patch Testing Guidelines*, K.E.Malten, W.G.van Ketel and J.P.Nater, 215
  - *Practical Dermatology*, I.B.Sneddon and R.E. Church, 3rd edition, 106
  - *Progress in Allergy*, P.Kallos, B.H.Waksman and A. de Weck, 682
  - *Rational Diagnosis and Treatment*, H.R.Wulff, 216
  - *Self-Assessment of Current Knowledge in Allergy*, Frazier, 685
  - *Skin Care*, B. Solomons, 455
  - *The Acnes: Clinical Features, Pathogenesis and Treatment*, W.J.Cunliffe and J.A.Cotterill, 684
  - *The Management of the Menopause and Post-Menopausal Years*, S.Campbell, 684
  - *The Molecular Biology of Skin*, P.D.Mier and D.W.K.Cotton, 109
  - *The Swollen Leg. Causes and Treatment*, M.D. Johnson and J. Pflug, 572
  - *The Vulva*, Constance M.Ridley, 215
  - *Varizen—Ulcus cruris und Thrombose*, K.Sigg, 455
  - *Veins and their Control*, J.T.Shepherd and P.M. Vanhoute, 217
- Brief Communications, 329
- British Association of Dermatologists: Annual Meeting, London 1976, Suppl. 14
- Bruises: painful bruising syndrome with vasculitis treated by plasmaphoresis (case report), Suppl. 14, 65
- Bullous lesions and psoriasis associated with generalized morphoea (case report), Suppl. 14, 33

- Canadian Dermatological Association: joint meeting with Irish Dermatological Society, 30th April–4th May 1976 in Dublin (brief communication), 330
- Carbohydrate metabolism in lichen planus, 9
- Carcinoma, basal cell—*see also* Ulcer, rodent
  - multiple; occurring in areas previously exposed to excessive amounts of diagnostic X-rays (case), 211
- β-Carotene in the treatment of discoid lupus erythematosus, 100
- Cathepsin A; first demonstration of in epidermis (brief communication), 675
- Cations, divalent; role of in epidermolysis, 25
- Cell cycle in psoriasis: a reappraisal, 577
- Cells:
  - epidermal cell cycle analysis; improved technique for (brief communication), 565
  - epidermal cells, human, undergoing keratinization; changes in dry weight and projected area of, as determined by scanning interference microscopy, 621
  - killer (K)-cells; characteristics and clinical importance of, 449
  - Langerhans cells: involvement in the pathogenesis of mycosis fungoides, 665
  - leukocyte chemotaxis in psoriasis scale, 1
  - leukocyte transfusion and plasmaphoresis in treatment of pyoderma gangrenosum with cellular immunity deficit (case report), Suppl. 14, 67
  - lymphocytes, human peripheral; effect of collagenase on surface markers of, 361
  - lymphocytes, thymus-dependent; deficiency in progressive systemic sclerosis, 469
  - lymphocytes, thymus-dependent; dissociated impairment of, in psoriasis, 295
  - skin cells, acquiring bacterial antigens; cytotoxic effect of antibacterial antibody on, in eczema, 493
  - sunburn cell in mouse skin: preliminary quantitative studies on its production, 459
- Cement, causing caustic ulcers, 547
- Cetomacrogol cream BPC (Formula A) with 1% cyproterone acetate; effect of on sebum excretion rate in patients with acne, 427
- Chloride in sweat; metal corrosion by, 417

Ciclopirox (new topical pyrodonium antimycotic agent) in treatment of superficial dermatomycoses, 83
Clomocycline in the treatment of persistent palmoplantar pustulosis; double-blind trial, 317
Coagulopathy, chronic intravascular; with ?pyoderma gangrenosum (case report), Suppl. 14, 70
Collagen synthesis; effect of hydrocortisone butyrate on (brief communication), 566
Collagenase; effect of on surface markers of human peripheral lymphocytes, 361
Comment, 449 665
Complement activation, alternative pathway: possible mechanism inducing skin lesions in benign gonococcal sepsis, 303
  component profiles in urticaria, dermatitis herpetiformis and alopecia areata, 145
Compositae oleoresins; airborne contact dermatitis from, simulating photodermatitis, 613
Computer, image analysing (Quantimet 720): practical applications of in investigative dermatology, 341
Connective tissues and physical training in young mice: biochemistry of skin, 525
Correspondence, 105
Corrosion of metal by chloride in sweat, 417
Corticosteroids:
  antibiotic/corticoid combination in the treatment of impetiginized atopic dermatitis; co-operative double-blind trial, 323
  effect of hydrocortisone butyrate on collagen synthesis (brief communication), 566
  glaucoma following application of corticosteroid to the skin of the eyelids, 207
  systemic corticosteroids in treatment of erythema multiforme in children, 181
Corticotrophin and vitiligo: preliminary observations (brief communication), 329
Crohn's disease, metastatic, 551
Cutis gyrata of face, with benign paraproteinaemia (case), 212
Cutis laxa and post-inflammatory elastolysis; actinic damage as possible inducer of (letter), 105
Cyproterone acetate 1% in Cetomacrogol cream BPC (Formula A); effect of on sebum excretion rate in patients with acne, 427

Dapsone toxicity, following high dosage, 79
de Lange syndrome (case report), Suppl. 14, 37
Degos' disease—*see* Papulosis, malignant atrophic
Dermatitis, atopic; impetiginized: double-blind trial of an antibiotic/corticoid combination in treatment of, 323
  psychological profile of patients with, 373
  contact: due to airborne Compositae oleoresins, simulating photodermatitis, 613
  cruris pustulosa et atrophicans; septrin in the treatment of, 71
  herpetiformis, urticaria and alopecia areata; complement component profiles in, 145
Dermatology, investigative: practical applications of an image analysing computer in, 341
Dermatomycoses, superficial; Ciclopirox in treatment of, 83
Dermatomyositis, juvenile (case report), Suppl. 14, 41
Dermatophytes; differentiation of by biochemical and serological methods (thesis summary), 453
  production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16
Dermatosis, bullous, of chronic renal failure, 541
  transient acantholytic (case report), Suppl. 14, 25
Dermis; measurement of steroid-induced atrophy of, using radiographic technique (summary), Suppl. 14, 12
Diphtheroids, aerobic cutaneous; taxonomy of (summary), Suppl. 14, 17
Dithranol and steroids; relapse rate of psoriasis treated with, 643
  local, with systemic use of a new retinoid in treatment of generalized psoriasis, 101
  versus triacetoxyanthracene: investigations *in vitro*, 193
DNA; excision repair of, in some photodermatoses, 13
Dowling Club, 209
Drugs, β-adrenergic blocking; adverse reaction to, 335
  appetite suppressant, and intestinal by-pass surgery in treatment of obesity, 219
Dyes, azo, aspirin and benzoates: follow-up study of patients with recurrent urticaria and hypersensitivity to, 19

Eczema—*see also* Dermatitis
  hypersensitivity to bacteria in. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci, 285. III. Arthus-like responses to bacterial antigens without specific antibody, 379. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens, 493
  infantile: long-term follow-up study, 365
  sociological implications of an epidemiological study in Birmingham, 137
Elastolysis, post-inflammatory, and cutis laxa; actinic damage as possible inducer of (letter), 105
Electron microscopy:
  histopathogenesis of pseudopyogenic granuloma in the light of ultrastructural studies (summary), Suppl. 14, 13
  lentigo maligna melanoma: an ultrastructural study, Suppl. 14, 14
  malignant melanoma: comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13
  necropsy and ultrastructural findings in histiocytic medullary reticulosis, 35
  studies of subcutaneous fat necrosis of the newborn, 407
  study of the permeability barrier in epidermis and oral epithelium using horseradish peroxidase as a tracer *in vitro*, 123
  study of tissue bound cyclic nucleotide phosphodiesterase in normal and psoriatic human skin, 591
  the role of divalent cations in epidermolysis, 25

Electron microscopy (*cont.*)
  ultrastructure of human epidermis following ultracentrifugation, 507
Electron microscopy, scanning: studies on the effect of salicylic acid on normal skin, 187
Enteropathy, gluten, and prurigo nodularis, 89
Enzymes:
  cathepsin A; first demonstration of in epidermis (brief communication), 675
  collagenase; effect of on surface markers of human peripheral lymphocytes, 361
  cyclic nucleotide phosphodiesterase, tissue bound; localization and activity of in normal and lack of changes in psoriatic human skin, 591
  enzyme cytochemical and immunocytological characterization of cutaneous lymphomas (summary), Suppl. 14, 9
  esterases, multiple; immunological demonstration of in human eccrine sweat, 67
  lysosomal hydrolases of the epidermis. 6. Changes in disease, 271
  lysosomal storage disorder of the epidermis characterized by deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627
Epicillin capsules; clinical and bacteriological evaluation of a twice daily regimen of, in treatment of skin infections, 177
Epidemiology of eczema in Birmingham; sociological implications of, 137
Epidermal cell cycle analysis; improved technique for (brief communication), 565
  s, human, undergoing keratinization; changes in dry weight and projected area of, as determined by scanning interference microscopy, 621
Epidermis and oral epithelium; study of the permeability barrier in, using horseradish peroxidase as a tracer *in vitro*, 123
  first demonstration of cathepsin A in (brief communication), 675
  human; ultrastructure of, following ultracentrifugation, 507
  in thyroid disease, 513
  lysosomal hydrolases of. 6. Changes in disease, 271
  lysosomal storage disorder of, characterized by deficiency of α-mannosidase and accumulation of mannose-rich materials, 627
  study of an inhibiting factor of epidermal proliferation in plucked skin and various tumours of mice, 131
Epidermolysis; role of divalent cations in, 25
Epidermophyton and *Trichophyton* species: production of antibiotics by (summary), Suppl. 14, 16
Epithelioma adenoides cysticum: genetic update, 225
  transformation of into multiple rodent ulcers: fact or fallacy, 233
Epithelium; causation of hyperproliferation of, in psoriasis (letter), 106
  oral, and epidermis; study of the permeability barrier in, using horseradish peroxidase as a tracer *in vitro*, 123
Erythema multiforme; histopathological spectrum of, 243
  in children: response to treatment with systemic corticosteroids, 181
Erythroderma and vasculitis (case report), Suppl. 14, 74
Esterases, multiple, in human eccrine sweat; immunological demonstration of, 67
Exfoliatin, staphylococcal; fate of in newborn and adult mice, 275

?Fasciitis, eosinophilic. ?Scleredema of Buschke (case report), Suppl. 14, 36
Fat necrosis, subcutaneous, of the newborn, 407
Fatty acids: changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats, 255
Fibrinolytic agents in the treatment of necrobiosis lipoidica (brief communication), 673
Fish odour syndrome (trimethylaminuria) in a child (summary), Suppl. 14, 11
Folliculitis, eosinophilic pustular, 661
Fungi, dermatophyte; differentiation of by biochemical and serological methods (thesis summary), 453

Glaucoma following application of corticosteroid to the skin of the eyelids, 207
Glucose-6-phosphate dehydrogenase deficiency and β-thalassaemia; leg ulcers in a family with, 203
Gluten enteropathy and prurigo nodularis, 89
Glycogen; PAS staining for in clinically normal psoriatic and non-psoriatic skin, 599
Grafts, pinch; management of stasis ulcers by (case report), Suppl. 14, 82
Granuloma annulare, cutaneous, and sarcoidosis: lymphokines (MIF) in the serum of patients with, 481
  direct immunofluorescence study, 487
  pseudopyogenic: histopathogenesis in the light of ultrastructural studies (summary), Suppl. 14, 13
Granulomatous disease, chronic (case report), Suppl. 14, 72

Hair, human; clinical importance of 'weathering' in, 111
Herniation of muscle of the lower legs (brief communication), 329
Herpes gestationis; further studies (summary), Suppl. 14, 19
Histochemistry: enzyme cytochemical and immunocytological characterization of cutaneous lymphomas, Suppl. 14, 9
Horseradish peroxidase, used as *in vitro* tracer in study of permeability barrier in epidermis and oral epithelium, 123
Hydrocortisone butyrate; effect of on collagen synthesis (brief communication), 566
  creams, proprietary: vasoconstrictor activities and bio-availabilities of 6 preparations, 423

Hydrolases, lysosomal, of the epidermis. 6. Changes in disease, 271
5-Hydroxytryptamine; hypersensitivity of cutaneous vascular smooth muscle to in scleroderma, 51
Hypersensitivity to aspirin, benzoates and azo dyes: follow-up study of patients with recurrent urticaria and, 19
  to bacteria in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci, 285. III. Arthus-like responses to bacterial antigens without specific antibody, 379. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens, 493
  to 5-hydroxytryptamine of cutaneous vascular smooth muscle in scleroderma, 51

Immunology:
  alternative pathway complement activation: possible mechanism inducing skin lesions in benign gonococcal sepsis, 303
  Arthus-like responses to bacterial antigens without specific antibody in eczema, 379
  auto-antibody profile in alopecia totalis and diffuse alopecia, 61
  complement component profiles in urticaria, dermatitis herpetiformis and alopecia areata, 145
  cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens in eczema, 493
  differentiation of dermatophytes by biochemical and serological methods (thesis summary), 453
  direct immunofluorescence study in granuloma annulare, 487
  effect of collagenase on surface markers of human peripheral lymphocytes, 361
  enzyme cytochemical and immunocytological characterization of cutaneous lymphomas (summary), Suppl. 14, 9
  generalized warts and immune deficiency, 559
  histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease, 631
  HL-A antigens in guttate psoriasis, 163
  HL-A antigens in lichen planus, 169
  HL-A antigens in vitiligo, 173
  immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295
  immunological demonstration of multiple esterases in human eccrine sweat, 67
  lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare, 481
  macrophage inhibitor factor in cutaneous lymphoproliferative diseases, 475
  studies in herpes gestationis (summary), Suppl. 14, 19
  titre and immunoglobulin class of antibodies to staphylococci and micrococci in eczema, 285
  T-lymphocyte deficiency in progressive systemic sclerosis, 469
Inflammation, ultraviolet-induced, in human skin; increase in prostaglandin E_2 and $F_{2\alpha}$ in exudate (summary), Suppl. 14, 21
Intestinal by-pass surgery and appetite suppressant drugs in treatment of obesity, 219
  flora of man; effect of long term tetracycline treatment for acne vulgaris on the occurrence of R factors in, 311
Irish Dermatological Society; joint meeting with Canadian Dermatological Association, Dublin, 30th April–4th May 1976 (brief communication), 330
Ischaemia, digital; due to vascular anomalies (summary), Suppl. 14, 20
Itching, paroxysmal, in multiple sclerosis, 555

Keratinization of human epidermal cells; changes in dry weight and projected area as determined by scanning interference microscopy, 621
Keratoacanthomas, multiple (case report), Suppl. 14, 78
Keratohyalin synthesis, bovine; kinetics of *in vitro*, 115
Killer (K)-cells; characteristics and clinical importance of, 449

Langerhans cells: involvement in the pathogenesis of mycosis fungoides, 665
Lecithin, epidermal; changes in transepidermal water loss and the composition of, after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats, 255
Lentigo maligna melanoma: ultrastructural study (summary), Suppl. 14, 14
Leucosis and skin: acute lymphoid immunoblastic leukaemia, 45
Leukaemia, acute lymphoid immunoblastic, with specific skin lesions, 49
  bullous pyoderma of (case), 209
  leukaemic transformation in mycosis fungoides (summary), Suppl. 14, 10
  multiple cutaneous malignancies in (case), 210
Leukocyte chemotaxis in psoriasis scale, 1
  transfusion and plasmaphoresis in treatment of pyoderma gangrenosum with cellular immunity deficit (case report), Suppl. 14, 67
Lichen aureus (case report), Suppl. 14, 45
  myxoedematosus (case report), Suppl. 14, 52
  planus; carbohydrate metabolism in, 9
    HL-A antigens in, 169
  striatus; pathology of (summary), Suppl. 14, 18
Lipodystrophia centrifugalis abdominalis infantilis (case report), Suppl. 14, 44
Livedo reticularis with thrombocythaemia in association with polycythaemia rubra vera (case report), Suppl. 14, 63
Lupus erythematosus, discoid: *β*-carotene in the treatment of, 100
  systemic: atrophie blanche lesions closely resembling malignant atrophic papulosis in, 649
Lymphocytes, human peripheral; effect of collagenase on surface markers of, 361
  thymus-dependent; deficiency in progressive systemic sclerosis, 469
  dissociated impairment of, in psoriasis, 295
Lymphokines: macrophage inhibitor factor in cutaneous lymphoproliferative diseases, 475

Lymphokines (*cont.*)
  macrophage inhibitor factor in serum of patients with sarcoidosis and cutaneous granuloma annulare, 481
Lymphomas, cutaneous; enzyme cytochemical and immunocytological characterization of (summary), Suppl. 14, 9
Lymphoproliferative diseases, cutaneous; macrophage inhibitor factor in, 475
Lysosomal storage disorder of epidermis; characterized by deficiency of α-mannosidase and accumulation of mannose-rich materials, 627

Macrophage inhibitor factor in cutaneous lymphoproliferative diseases, 475
  in serum of patients with sarcoidosis and cutaneous granuloma annulare, 481
Malignancies, cutaneous, multiple; in leukaemia (case), 210
α-Mannosidase, deficiency of, and accumulation of mannose-rich materials; characterizing a lysosomal storage disorder of the epidermis, 627
Melanoma: lentigo maligna melanoma—an ultrastructural study (summary), Suppl. 14, 14
  malignant: comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13
Metal corrosion by chloride in sweat, 417
8-Methoxypsoralen-black light therapy; effects and side-effects of in more than 1500 patients (summary), Suppl. 14, 21
  inducing bullous pemphigoid (brief communication), 568
Micrococci and staphylococci; titre and immunoglobulin class of antibodies to, in eczema, 285
Micro-organisms of human skin; studies on interactions of, on solid surfaces (thesis summary), 571
Microscopy, scanning interference: study of changes in dry weight and projected area of human epidermal cells undergoing keratinization, 621
Molluscum contagiosum with giant lesions (case), 213
Morphoea, acute widespread (case report), Suppl. 14, 34
  generalized; with bullous lesions and psoriasis (case report), Suppl. 14, 33
Mudi-chood on the pinnae, 197
Muscle, cutaneous vascular smooth; hypersensitivity of to 5-hydroxytryptamine in scleroderma, 51
  herniation of the lower legs (brief communication), 329
Mycosis fungoides; involvement of Langerhans cells in the pathogenesis of, 665
  psoriasis and parapsoriasis; study of efficacy of photochemotherapy in treatment of (summary), Suppl. 14, 20
  with leukaemic transformation (summary), Suppl. 14, 10

Naevi, pigmented, multiple; in an albino, 199
Naevus, bathing trunk; with neoplastic transformation (case report), Suppl. 14, 77
  epidermal, unilateral; resembling acanthosis nigricans, 433
  non-acne: clinical and laboratory study (summary), Suppl. 14, 18
  syndrome, epidermal, 647
Nail dystrophy, psoriatic; treated with intradermal steroid injections, 75
Nandrolone; diffusion of through hydrated human cadaver skin, 265
Necrobiosis lipoidica; fibrinolytic agents in the treatment of (brief communication), 673
Necrosis of subcutaneous fat in the newborn, 407
Neoplastic transformation of bathing trunk naevus (case report), Suppl. 14, 77
Nerve supply as a stimulator of the growth of tissues, including skin (summary), Suppl. 14, 9
News and Notices, 113, 223, 339, 457, 575 686
Nucleotide phosphodiesterase, cyclic, tissue bound; localization and activity of in normal and lack of changes in psoriatic human skin, 591

Obesity; appetite suppressant drugs and intestinal by-pass surgery in treatment of, 219
Obituaries:
  Dowling, G.B., 677
  Farb, M., 681
  Gunther, W.W., 681
  Reed, W.B., 681
  Savatard, J., 681
Oleoresins, Compositae; airborne contact dermatitis from, simulating photodermatitis, 613

Papulosis, malignant atrophic; close resemblance of atrophie blanche lesions to, in systemic lupus erythematosus, 649
Paraproteinaemia, benign; associated with cutis gyrata of face (case), 212
Parapsoriasis, psoriasis and mycosis fungoides; study of efficacy of photochemotherapy in treatment of (summary), Suppl. 14, 20
Pemphigoid, bullous, and psoriasis: does subclinical bullous pemphigoid exist?, 535
  juvenile (case report), Suppl. 14, 23
  PUVA-induced (brief communication), 568
  localized, 531; (case report) Suppl. 14, 26
Pemphigus induced by rifampicin, 445
Pemphigus-like syndrome induced by D-penicillamine, 99
D-Penicillamine inducing pemphigus-like syndrome, 99
Periodic-acid-Schiff staining for glycogen in clinically normal psoriatic and non-psoriatic skin, 599
Permeability barrier; study of in epidermis and oral epithelium using horseradish peroxidase as a tracer *in vitro*, 123
Phosphorus, sodium and potassium content of normal and psoriatic skin (brief communication), 674
Photobiology: sunburn cell in mouse skin; preliminary quantitative studies on production, 459

Photochemotherapy; study of efficacy in 50 patients with psoriasis and other dermatoses (summary), Suppl. 14, 20
Photodermatitis; simulated by airborne contact dermatitis from Compositae oleoresins, 613
Photodermatoses; excision of repair of DNA in, 13
Photosensitizers and ultraviolet irradiation; effects of on the biosynthesis and metabolism of prostaglandins, 397
Physical training and connective tissues in young mice: biochemistry of skin, 525
Piebaldism (case report), Suppl. 14, 43
Pigmented lesions in newborn infants, 389
Pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153
Plasma volume, intravascular albumin and its transcapillary escape rate in patients with extensive skin disease, 519
Plasmaphoresis and leukocyte transfusion in treatment of pyoderma gangrenosum with cellular immunity deficit (case report), Suppl. 14, 67
  in treatment of painful bruising syndrome with vasculitis (case report), Suppl. 14, 65
Polychondritis, relapsing (case report), Suppl. 14, 81
Polycythaemia rubra vera; associated with livedo reticularis with thrombocythaemia (case report), Suppl. 14, 63
Porokeratosis, disseminated superficial actinic, 429
Porphyria cutanea tarda (case report), Suppl. 14, 28
Potassium, sodium and phosphorus content of normal and psoriatic skin (brief communication), 674
Practolol reaction; mechanism of, 337
Prostaglandin E_2 and $F_{2\alpha}$; increase in ultraviolet-induced inflammation in human skin (summary), Suppl. 14, 21
  effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of, 397
Proteinosis, lipoid (case report), Suppl. 14, 48
Protoporphyria, erythropoietic: hepatic implications, 157
Prurigo, nodular (case report), Suppl. 14, 79
  and gluten enteropathy, 89
Psoriasis and bullous lesions associated with generalized morphoea (case report), Suppl. 14, 33
  and bullous pemphigoid: does subclinical bullous pemphigoid exist?, 535
  and other dermatoses; study of efficacy of photochemotherapy in treatment of (summary), Suppl. 14, 20
  bacterial flora in, 603
  causation of epithelial hyperproliferation in (letter), 106
  cell cycle in, 577
  generalized; systemic use of a new retinoid in, with and without local dithranol treatment, 101
  guttate; HL-A antigens in, 163
  immunological aspects of. II. Dissociated impairment of thymus-dependent lymphocytes, 295
  in vitro evaluation of antipsoriatic activity of dithranol (anthralin) and triacetoxyanthracene, 193
  localization and activity of tissue bound cyclic nucleotide phosphodiesterase in normal and lack of changes in psoriatic human skin, 591
  PAS staining for glycogen in clinically normal psoriatic and non-psoriatic skin, 599
  scale; leukotactic properties of soluble substances in, 1
  sodium, potassium and phosphorus content of normal and psoriatic skin (brief communication), 674
  treatment of nail dystrophy in, with intradermal steroid injections, 75
  worsened by adding steroids to a dithranol regime: relapse rate of, 643
Purpura, benign hyperglobulinaemic, of Waldenstrom (case report), Suppl. 14, 60
Pustulosis, persistent palmoplantar; double-blind trial of clomocycline in the treatment of, 317
PUVA—*see* 8-Methoxypsoralen-black light therapy
Pyoderma gangrenosum, bullous, of leukaemia (case), 209
  with cellular immunity deficit treated with plasmaphoresis and leukocyte transfusion (case report), Suppl. 14, 67
  with chronic intravascular coagulopathy (case report), Suppl. 14, 70
  with hypoplastic anaemia and tabes dorsalis (case report), Suppl. 14, 69

Quantimet 720 (image analysing computer); practical applications of in investigative dermatology, 341

R factors in the intestinal flora of man; effect of long term tetracycline treatment for acne vulgaris on the occurrence of, 311
Radiographic technique in measurement of steroid-induced dermal atrophy (summary), Suppl. 14, 12
Renal failure, chronic; bullous dermatosis associated with, 541
Reticulosis, histiocytic medullary; necropsy and ultrastructural findings in, 35
  secondary syphilis with histology simulating, 251
Retinoid, new; systemic use of with and without local dithranol treatment in generalized psoriasis, 101
Reviews, 111, 219, 335
Rifampicin; inducing pemphigus, 445

Salicylic acid; effect of on normal skin, 187
Sarcoidosis (case report), Suppl. 14, 56
  and cutaneous granuloma annulare; lymphokines (MIF) in the serum of patients with, 481
  presenting as erythroderma with keratotic spines and palmar pits, in a child, 93
Schistosomiasis, cutaneous ectopic (case report), Suppl. 14, 57
?Scleredema of Buschke. ?Eosinophilic fasciitis (case report), Suppl. 14, 36
Scleroderma; hypersensitivity of cutaneous vascular smooth muscle to 5-hydroxytryptamine in, 51
Sclerosis, multiple; paroxysmal itching in, 555
  systemic; prognosis of, 57

Sclerosis (*cont.*)
  progressive: thymus-dependent lymphocyte deficiency in, 469
Sebum excretion rate in patients with acne; effect of 1% cyproterone acetate in Cetomacrogol cream BPC (Formula A) on, 427
Sepsis, benign gonococcal; alternative pathway complement activation as a possible mechanism inducing skin lesions in, 303
Septrin in the treatment of dermatitis cruris pustulosa et atrophicans, 71
Sézary syndrome (case report), Suppl. 14, 75
Skin disease, extensive; plasma volume, intravascular albumin and its transcapillary escape rate in patients with, 519
  human, and leucosis: acute lymphoid immunoblastic leukaemia, 45
  human, cadaver, hydrated; diffusion of nandrolone through, 265
    clinically normal psoriatic and non-psoriatic; PAS staining for glycogen in, 599
    effects of occlusion upon population dynamics of bacteria of, 607
    in vitro studies of antagonism among bacteria of (thesis summary), 571
    increase in prostaglandin E_2 and $F_{2\alpha}$ in ultraviolet-induced inflammation in (summary), Suppl. 14, 21
    localization and activity of tissue bound cyclic nucleotide phosphodiesterase in normal and lack of changes in psoriatic, 591
    micro-organisms of: studies on interactions of on solid surfaces (thesis summary), 571
    normal: effect of salicylic acid on, 187
  infections: clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules in the treatment of, 177
  lesions in benign gonococcal sepsis; alternative pathway complement activation as a possible mechanism of induction, 303
  mouse: preliminary quantitative studies on production of sunburn cell in, 459
Sodium, potassium and phosphorus content of normal and psoriatic skin (brief communication), 674
Solar damage: possible inducer of post-inflammatory elastolysis and cutis laxa (letter), 105
Staphylococci and micrococci; titre and immunoglobulin class of antibodies to, in eczema, 285
Steroid injections, intradermal; in treatment of psoriatic nail dystrophy, 75
  and dithranol; relapse rate of psoriasis treated with, 643
  topical; measurement of dermal atrophy induced by, using a radiographic technique (summary), Suppl. 14, 12
Stevens-Johnson syndrome—*see* Erythema multiforme
Sunburn cell in mouse skin: preliminary quantitative studies on its production, 459
Sweat, eccrine, human: immunological demonstration of multiple esterases in, 67
  metal corrosion by chloride in, 417
Sycosis, lupoid (case report), Suppl. 14, 59
Syphilis, secondary; histology simulating reticulosis in, 251

Tabes dorsalis associated with pyoderma gangrenosum and hypoplastic anaemia (case report), Suppl. 14, 69
Tetracycline treatment, long term, for acne vulgaris; effect of on occurrence of R factors in the intestinal flora of man, 311
β-Thalassaemia and glucose-6-phosphate dehydrogenase deficiency; leg ulcers in a family with, 203
Thesis summaries, 453, 571
Thrombocythaemia with livedo reticularis in association with polycythaemia rubra vera (case report), Suppl. 14, 63
Thymus-dependent (T) lymphocyte deficiency in progressive systemic sclerosis, 469
Thyroid disease; epidermis in, 513
Tissue growth; nerve supply as a stimulator of (summary), Suppl. 14, 9
Treatment:
  antibiotic/corticoid combination in impetiginized atopic dermatitis (double-blind trial), 323
  appetite suppressant drugs and intestinal by-pass surgery in obesity, 219
  β-carotene in discoid lupus erythematosus, 100
  Ciclopirox (new topical pyrodonium antimycotic) in superficial dermatomycoses, 83
  clomocycline in persistent palmoplantar pustulosis, 317
  corticosteroids, systemic, in erythema multiforme in children, 181
  corticotrophin in vitiligo (brief communication), 329
  dapsone in dermatitis herpetiformis; high dose toxicity, 79
  epicillin capsules in skin infections, 177
  fibrinolytic agents in necrobiosis lipoidica (brief communication), 673
  pinch grafts in stasis ulcers (case report), Suppl. 14, 82
  plasmaphoresis and leukocyte transfusion in pyoderma gangrenosum with cellular immunity deficit (case report), Suppl. 14, 67
  plasmaphoresis in painful bruising syndrome with vasculitis (case report), Suppl. 14, 65
  retinoid, new systemic, with and without local dithranol in generalized psoriasis, 101
  septrin in dermatitis cruris pustulosa et atrophicans, 71
  steroid injections, intradermal, in psoriatic nail dystrophy, 75
  steroids and dithranol in psoriasis; relapse rate differences, 643
Triacetoxyanthracene versus dithranol (anthralin): investigations *in vitro*, 193
Trichophyton and *Epidermophyton* species; production of antibiotics by (summary), Suppl. 14, 16
Tricho-rhino-phalangeal syndrome (case report), Suppl. 14, 39
Triglycerides, pure fatty acid; changes in transepidermal water loss and composition of epidermal lecithin after applications of to the skin of essential fatty acid-deficient rats, 255

Trimethylaminuria (fish odour syndrome) in a child (summary), Suppl. 14, 11
Tuberculosis, cutaneous, of nose with unusual clinical and histological features (summary), Suppl. 14, 15
Tumours, various, and plucked skin of mice; study of an inhibiting factor of epidermal proliferation in, 131

Ulcers, caustic; caused by cement, 547
  leg: in a family with both beta-thalassaemia and glucose-6-phosphate dehydrogenase deficiency, 203
  rodent—*see also* Carcinoma, basal cell
    multiple; transformation of epithelioma adenoides cysticum into: fact or fallacy, 233
  stasis; management of by pinch graft (case report), Suppl. 14, 82
Ultraviolet irradiation and photosensitizers; effects of on the biosynthesis and metabolism of prostaglandins, 397
  and psoralens in the treatment of psoriasis—*see* 8-Methoxypsoralen-black light therapy
  in the production of the sunburn cell in mouse skin, 459
  inflammation of human skin induced by; increase in prostaglandin E_2 and $F_{2\alpha}$ in exudate, Suppl. 14, 21
Uroporphyria, erythropoietic; late onset (case report), Suppl. 14, 30
Urticaria, dermatitis herpetiformis and alopecia areata; complement component profiles in, 145
  recurrent, and hypersensitivity to aspirin, benzoates and azo dyes; follow-up study of patients with, 19

Vascular anomalies causing digital ischaemia (summary), Suppl. 14, 20
Vasculitis and erythroderma (case report), Suppl. 14, 74
  associated with painful bruising syndrome, treated by plasmaphoresis (case report), Suppl. 14, 65
Vasoconstrictor activities of 6 proprietary hydrocortisone creams, 423
Vitamin K1; adverse skin reaction from, 653
Vitiligo and corticotrophin: preliminary observations (brief communication), 329
  HL-A antigens in, 173

Warts, generalized, and immune deficiency, 559
Water loss, transepidermal, and composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats, 255

Xanthoma, plane, generalized (case report), Suppl. 14, 50
Xeroderma pigmentosum in brother and sister (case report), Suppl. 14, 47
X-rays, diagnostic; multiple basal cell carcinomata occurring in areas previously exposed to excessive amounts of (case), 211

AUTHORS

ABDEL-AZIZ, A.M.; ATTIA, M.K. and: epidermal naevus syndrome, 647
AL-AHMAR, H.F. and KURBAN, A.K., psychological profile of patients with atopic dermatitis, 373
ALMEYDA, J.; BRIGDEN, W.D. and: adverse reaction to the β-adrenergic blocking drugs, 335
ALY, R., MAIBACH, H.I. and MANDEL, A., bacterial flora in psoriasis, 603
ANDERSON, D.E. and HOWELL, J.B., epithelioma adenoides cysticum: genetic update, 225
ANDERSON, D.E.; HOWELL, J.B. and: transformation of epithelioma adenoides cysticum into multiple rodent ulcers: fact or fallacy, 233
ATHERTON, D.J. and WELLS, R.S., ?scleredema of Buschke. ?Eosinophilic fasciitis (case report), Suppl. 14, 36
ATTIA, M.K. and ABDEL-AZIZ, A.M., epidermal naevus syndrome, 647
AZIZ, A.A., SOLIMAN, M., SHOEIB, S., TADROS, S.S.; EL ZAWAHRY, M. and: clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules for the treatment of skin infections, 177
BAKER, H., WARIN, A.; KLABER, M. and: porphyria cutanea tarda (case report), Suppl. 14, 28
BARNES, H.M. and SARKANY, I., adverse skin reaction from vitamin K1, 653
BARRY, B.W. and WOODFORD, R., proprietary hydrocortisone creams, 423
BAUER, F.W. and DE GROOD, R.M., improved technique for epidermal cell cycle analysis (brief communication), 565
BEDI, T.R. and PINKUS, H., histopathological spectrum of erythema multiforme, 243
BELCHER, R.W., WINKELMANN, R.K.; UMBERT, P. and: lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare, 481
BELCHER, R.W., WINKELMANN, R.K.; UMBERT, P. and: macrophage inhibitor factor (MIF) in cutaneous lymphoproliferative diseases, 475
BENTLEY-PHILLIPS, C.B. and WELLS, G.C., transient acantholytic dermatosis (case report), Suppl. 14, 25

Bergers, A.M.G., van den Hurk, J.J.M.A., Mier, P.D., van de Staak, W.J.B.M.; Mali, J.W.H. and: a lysosomal storage disorder of the epidermis characterized by a deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627

Bernengo, M.G., Zina, A. and Zina, G., leucosis and skin: acute lymphoid immunoblastic leukaemia, 45

Berrens, L., Jankowski, E. and Jankowski-Berntsen, I., complement component profiles in urticaria, dermatitis herpetiformis and alopecia areata, 145

Besser, F. and Wells, R.S., the tricho-rhino-phalangeal syndrome (case report), Suppl. 14, 39

Betuel, H., Ortonne, J.P., Thivolet, J.; Retornaz, G. and; HL-A antigens and vitiligo, 173

Bibel, D.J, Lovell, D.J. and Smiljanic, R.J., effects of occlusion upon population dynamics of skin bacteria, 607

Black, M.M.; Carruthers, J.A. and: further studies in herpes gestationis (summary), Suppl. 14, 19

Black, M.M. and Hudson, P.M., atrophie blanche lesions closely resembling malignant atrophic papulosis (Degos' disease) in systemic lupus erythematosus, 649

Black, M.M.; Hudson, P.M. and: nodular prurigo (case report), Suppl 14, 79

Black, M M., Sparkes, C.G.; James, M.P. and: measurement of dermal atrophy induced by topical steroids using a radiographic technique (summary), Suppl. 14, 12

Bleehen, S.S., Edwards, I.R. and Clark, R.G., treatment of obesity: appetite suppressant drugs and intestinal by-pass surgery, 219

Bleehen, S.S., Hartley, Lisa and Senior, Jennifer: lentigo maligna melanoma: an ultrastructural study (summary), Suppl. 14, 14

Bleehen, S.S., Zaynoun, S., Patterson, W.D., MacKie, Rona M., Cochran, A.J.; Hunter, J.A.A. and: malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13

Bowker, N.C., Cross, K.W., Fairburn, E.A. and Wall, M., sociological implications of an epidemiological study of eczema in the City of Birmingham, 137

Braun-Falco, O. and Burg, G., enzyme cytochemical and immunocytological characterization of cutaneous lymphomas (summary), Suppl. 14, 9

Braun-Falco, O.; Scherer, R. and: alternative pathway complement activation: a possible mechanism inducing skin lesions in benign gonococcal sepsis, 303

Brigden, W.D. and Almeyda, J., adverse reaction to the β-adrenergic blocking drugs, 335

Brochier, J., Meynadier, J., Clot, J., Charmasson, E., Dardenne, M.; Guilhou, J.J. and: immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295

Brookes, D.B., Pye, R.J ; Burton, J L. and: metal corrosion by chloride in sweat, 417

Bullen, M.F., Cudworth, A.G., Clough, S.A.; Lowe, N.J. and; carbohydrate metabolism in lichen planus, 9

Burg, G.; Braun-Falco, O. and: enzyme cytochemical and immunocytological characterization of cutaneous lymphomas (summary), Suppl. 14, 9

Burns, D.A. and Sarkany, I., generalized morphoea with bullous lesions and psoriasis (case report), Suppl. 14, 33

Burns, D.A. and Sarkany, I., management of stasis ulcers by pinch graft (case report), Suppl 14, 82

Burton, J L., bullous pyoderma of leukaemia (case), 209

Burton, J.L., Harris, J.I.; Pye, R.J. and: effect of 1% cyproterone acetate in Cetomacrogol cream BPC (Formula A) on sebum excretion rate in patients with acne, 427

Burton, J.L.; Pye, R.J. and: multiple cutaneous malignancies in leukaemia (case), 210

Burton, J.L., Pye, R.J. and Brookes, D.B., metal corrosion by chloride in sweat, 417

Byrne, J.P.H. and Marten, R.H., lichen myxoedematosus (case report), Suppl. 14, 52

Byrne, J.P.H. and Williams, D.I., bisalbuminaemia (case report), Suppl. 14, 54

Byrne, J.P.H. and Williams, D.I., case for diagnosis (case report), Suppl. 14, 83

Cairns, R.J., lipodystrophia centrifugalis abdominalis infantilis (case report), Suppl. 14, 44

Calnan, C.D., Canadian–Irish dermatological meeting (brief communication), 330

Carruthers, J.A. and Black, M.M., further studies in herpes gestationis (summary), Suppl. 14, 19

Carruthers, J.A. and Greaves, M.W., chronic granulomatous disease (case report), Suppl. 14, 72

Champion, R.H., Welbourn, Esther; Parish, W.E. and: hypersensitivity to bacteria in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci, 285. III. Arthus-like responses to bacterial antigens without specific antibody, 379. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens, 493

Charmasson, E., Meynadier, J., Clot, J., Dardenne, M., Brochier, J.; Guilhou, J.J. and: immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295

Clark, R.G., Edwards, I.R.; Bleehen, S.S. and: treatment of obesity: appetite suppressant drugs and intestinal by-pass surgery, 219

Clarke, Jacqueline, and Wells, G.C., livedo reticularis with thrombocythaemia in association with polycythaemia rubra vera (case report), Suppl. 14, 63

Clayton, R. and Feiwel, M., benign hyperglobulinaemic purpura of Waldenstrom (case report), Suppl. 14, 60

Clayton, R., Feiwel, M. and Valdimarsson, H., pyoderma gangrenosum with cellular immunity deficit treated with plasmaphoresis and leukocyte transfusion (case report), Suppl. 14, 67

Clayton, Yvonne M., Youssef, Nagwan, Holt, G., Wyborn, C.; Noble, W.C. and: the production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16

Clot, J., Meynadier, J., Charmasson, E., Dardenne, M., Brochier, J.; Guilhou, J.J. and: immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295

Clough, S.A., Cudworth, A.G., Bullen, M.F.; Lowe, N.J. and: carbohydrate metabolism in lichen planus, 9

Cochran, A.J., Zaynoun, S., Patterson, W.D., MacKie, Rona M., Bleehen, S.S.; Hunter, J.A.A. and: malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13

Cochran, Rebecca E.I., Thomson, J., Fleming, K.A. and Strong, Alexandra M.M., histology simulating reticulosis in secondary syphilis, 251

Cochran, Rebecca E.I., Thomson, J. and MacSween, R.N.M., an auto-antibody profile in alopecia totalis and diffuse alopecia, 61

Cohen, T.; Ganor, S. and: leg ulcers in a family with both beta-thalassaemia and glucose-6-phosphate dehydrogenase deficiency, 203

Comaish, J.S., Hassanyeh, F., Stephens, A.; Goolamali, S.K. and: familial Behçet's syndrome, 637

Copeman, P.W.M.; Staughton, R.C.D. and: chronic intravascular coagulopathy with ?pyoderma gangrenosum (case report), Suppl. 14, 70

Corbett, Margaret F., Hanna, M.J.; Ward, Joanna M. and: a double-blind trial of clomocycline in the treatment of persistent palmoplantar pustulosis, 317

Cowen, T., Wilson Jones, E.; Eady, R.A.J. and: pseudopyogenic granuloma: the histopathogenesis in the light of ultrastructural studies (summary), Supp. 14, 13

Cox, A.J., Jacobs, A.H.; Walton, R.G. and: pigmented lesions in newborn infants, 389

Cross, K.W., Fairburn, E.A., Wall, M.; Bowker, N.C. and: sociological implications of an epidemiological study of eczema in the City of Birmingham, 137

Cubey, R.B., glaucoma following the application of corticosteroid to the skin of the eyelids, 207

Cudworth, A.G., Clough, S.A., Bullen, M.F.; Lowe, N.J. and: carbohydrate metabolism in lichen planus, 9

Cudworth, A.G., Woodrow, J.C.; Lowe, N.J. and: HL-A antigens in lichen planus, 169

Cunliffe, W.J., Hodgins, M.B. and Ead, R., a non-acne naevus. A clinical and laboratory study (summary), Suppl. 14, 18

Cunliffe, W.J., Perera, W.D.H., Thackray, P., Williams, M., Forster, R.A. and Williams, S.M., pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153

Curth, Helen O., unilateral epidermal naevus resembling acanthosis nigricans, 433

D'Angelo, I., Eusebi, V.; Hall, M. and: multiple pigmented naevi in an albino, 199

Danks, D., Greaves, M.W., Plummer, V.; Marks, R. and: trimethylaminuria or fish odour syndrome in a child (summary), Suppl. 14, 11

Dardenne, M., Meynadier, J., Clot, J., Charmasson, E., Brochier, J.; Guilhou, J.J. and: immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295

Davies, M. and Marks, R., studies on the effect of salicylic acid on normal skin, 187

De Grood, R.M.; Bauer, F.W. and: improved technique for epidermal cell cycle analysis (brief communication), 565

Devitt, H., Forgie, M.A.; Picton, W. and: practical applications of the quantimet 720, an image analysing computer, in the field of investigative dermatology, 341

Dimond, R.L., Erickson, K.L. and Wuepper, K.D., the role of divalent cations in epidermolysis, 25

Dodd, Janis, Holt, Shirley, Rowell, N.R.; Hughes, P. and: thymus-dependent (T) lymphocyte deficiency in progressive systemic sclerosis, 469

Ead, R., Hodgins, M.B., Cunliffe, W.J. and: a non-acne naevus. A clinical and laboratory study (summary), Suppl. 14, 18

Eady, R.A.J., Cowen, T. and Wilson Jones, E., pseudopyogenic granuloma: the histopathogenesis in the light of ultrastructural studies (summary), Suppl. 14, 13

Edwards, C.O., Rhodes, E.L., Powell, M.E.A.; Gange, R.W. and: pemphigus induced by rifampicin, 445

Edwards, I.R., Clark, R.G.; Bleehen, S.S. and: treatment of obesity: appetite suppressant drugs and intestinal by-pass surgery, 219

El Zawahry, M., Aziz, A.A., Soliman, M., Shoeib, S. and Tadros, S.S., clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules for the treatment of skin infections, 177

Elias, P.M., Klingler, W.G. and Montague, P.M., kinetics of *in vitro* bovine keratohyalin synthesis, 115

Elias, P.M., Varga, J.; Fritsch, P. and: the fate of staphylococcal exfoliatin in newborn and adult mice, 275

Elvy, B.L., Stubbing, D.G.; McKenzie, A.W. and: prurigo nodularis and gluten enteropathy, 89

Erickson, K.L., Wuepper, K.D.; Dimond, R.L. and: the role of divalent cations in epidermolysis, 25

Eusebi, V., D'Angelo, I.; Hall, M. and: multiple pigmented naevi in an albino, 199

Fairburn, E.A., Cross, K.W., Wall, M.: Bowker, N.C. and: sociological implications of an epidemiological study of eczema in the City of Birmingham, 137

Farr, M.; Keczkes, K. and: bullous dermatosis of chronic renal failure, 541

Feiwel, M.; Clayton, R. and: benign hyperglobulinaemic purpura of Waldenstrom (case report), Suppl. 14, 60
Feiwel, M., Valdimarsson, H., Clayton, R. and: pyoderma gangrenosum with cellular immunity deficit treated with plasmaphoresis and leukocyte transfusion (case report), Suppl. 14, 67
Ferrando, J., Tomás, J.M., Mieras, C., Peyrí, J.: Pinol-Aguadé, J. and: necropsy and ultrastructural findings in histiocytic medullary reticulosis, 35
Findlay, G.H. and Liebenberg, N.v.d.W., the ultrastructure of the human epidermis following ultracentrifugation, 507
Fleming, K.A., Thomson, J., Strong, Alexandra M.M.; Cochran, Rebecca E.I. and: histology simulating reticulosis in secondary syphilis, 251
Foreman, M.I. and Kelly, I., the diffusion of nandrolone through hydrated human cadaver skin, 265
Forgie, M.A., Devitt, H.; Picton, W. and: practical applications of the quantimet 720, an image analysing computer, in the field of investigative dermatology, 341
Forster, R.A., Perera, W.D.H., Thackray, P., Williams, M., Williams, S.M.; Cunliffe, W.J. and: pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153
Frain-Bell, W., Gould, P., Johnson, B.E., Mackenzie, L.A.; Laksmipathi, T. and: photochemotherapy. A study of its efficacy in fifty patients suffering from psoriasis and other dermatoses (summary), Suppl. 14, 20
Fraser, N.G., Kernohan, I.R.; Reid, T.M.S. and: generalized warts and immune deficiency, 559
Friedman-Birnbaum, R., Sobel, J.D., Lichtig, C.; Haim, S. and: histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease, 631
Frindel, E., Hewitt, J., Tubiana, M.; Guigon, M. and: study of an inhibiting factor of epidermal proliferation in plucked skin and various tumours of mice, 131
Fritsch, P., Elias, P. and Varga, J., the fate of staphylococcal exfoliatin in newborn and adult mice, 275

Gange, R.W., Rhodes, E.L., Edwards, C.O. and Powell, M.E.A., pemphigus induced by rifampicin, 445
Ganor, S. and Cohen, T., leg ulcers in a family with both beta-thalassaemia and glucose-6-phosphate dehydrogenase deficiency, 203
Gelfant, S., the cell cycle in psoriasis: a reappraisal, 577
Gokhale, B.B. and Gokhale, Tara B., corticotrophin and vitiligo: preliminary observations (brief communication), 329
Gold, S.C.; James, M.P. and: generalized plane xanthoma (case report), Suppl. 14, 50
Goldstein, D.J.; Yardley, H.J. and: changes in dry weight and projected area of human epidermal cells undergoing keratinization as determined by scanning interference microscopy, 621
Goldwein, M.I.; Shelley, W.B. and: high dose dapsone toxicity, 79
Goldyne, M.E., Linscheid, R.L.; Winkelmann, R.K. and: hypersensitivity of scleroderma cutaneous vascular smooth muscle to 5-hydroxytryptamine, 51
Goolamali, S.K., Comaish, J.S., Hassanyeh, F. and Stephens, A., familial Behçet's syndrome, 637
Gould, P., Johnson, B.E., Mackenzie, L.A., Frain-Bell, W.; Laksmipathi, T. and: photochemotherapy. A study of its efficacy in fifty patients suffering from psoriasis and other dermatoses (summary), Suppl. 14, 20
Gray, W.M.; McCallum, D.I. and: metastatic Crohn's disease, 551
Greaves, M.W.; Carruthers, J.A. and: chronic granulomatous disease (case report), Suppl. 14, 72
Greaves, M.W., Danks, D., Plummer, V.; Marks, R. and: trimethylaminuria or fish odour syndrome in a child (summary), Suppl. 14, 11
Greaves, M.W., Hensby, C.N., Pazzaglia, A., Plummer, N.; Kobza Black, A. and: increase in prostaglandin E_2 and $F_{2\alpha}$ in inflammatory exudate at 4–48 h after UVB irradiation of human skin (summary), Suppl. 14, 21
Greaves, M.W.; MacDonald, D.M. and: Cornelia de Lange syndrome (case report), Suppl. 14, 37
Greaves, M.W., Morrison, J.G.L.; MacDonald, D.M. and: cutaneous ectopic schistosomiasis (case report), Suppl. 14, 57
Greaves, M.W.; Skeete, M. and: juvenile bullous pemphigoid (case report), Suppl. 14, 23
Griffiths, W.A.D., reticulate acropigmentation of Kitamura, 437
Guigon, M., Frindel, E., Hewitt, J. and Tubiana, M., study of an inhibiting factor of epidermal proliferation in plucked skin and various tumours of mice, 131
Guilhou, J.J., Meynadier, J., Clot, J., Charmasson, E., Dardenne, M. and Brochier, J., immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295

Habbig, J.; Herrmann, W.P. and: immunological demonstration of multiple esterases in human eccrine sweat, 67
Haim, S., Sobel, J.D., Friedman-Birnbaum, R. and Lichtig, C., histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease, 631
Hajini, H., Hussain, S.T. and Shah, S.N.A., sodium, potassium and phosphorus content of normal and psoriatic skin (brief communication), 674
Hall, M., Eusebi, V. and D'Angelo, I., multiple pigmented naevi in an albino, 199
Hamilton, E.B.D., juvenile dermatomyositis (case report), Suppl. 14, 41
Hanna, M.J., Corbett, Margaret F.; Ward, Joanna M. and: a double-blind trial of clomocycline in the treatment of persistent palmoplantar pustulosis, 317

Hannuksela, M., Suhonen, R. and Karvonen, J., caustic ulcers caused by cement, 547

Harman, R.R.M., cutis gyrata of the face with benign paraproteinaemia (case), 212

Harman, R.R.M., Pye, R.J.; Peachey, R.D.G. and: the treatment of psoriatic nail dystrophy with intradermal steroid injections, 75

Harman, R.R.M., Warin, R.P.; Matthews, C.N. and: multiple basal cell carcinomata occurring in areas previously exposed to excessive amounts of diagnostic X-rays in 3 patients (case), 211

Harrington, Christine I., mycosis fungoides with leukaemic transformation (summary), Suppl. 14, 10

Harris, J.I., Burton, J.L.; Pye, R.J. and: effect of 1% cyproterone acetate in Cetomacrogol cream BPC (Formula A) on sebum excretion rate in patients with acne, 427

Hartley, Lisa, Senior, Jennifer; Bleehen, S.S. and: lentigo maligna melanoma: an ultrastructural study (summary), Suppl. 14, 14

Hartop, P.J. and Prottey, C., changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats, 255

Hassanyeh, F., Comaish, J.S., Stephens, A.; Goolamali, S.K. and: familial Behçet's syndrome, 637

Heikkinen, E.; Kiiskinen, A. and: physical training and connective tissues in young mice, 525

Hensby, C.N., Greaves, M.W., Pazzaglia, A., Plummer, N.; Kobza Black, A. and: increase in prostaglandin E_2 and $F_{2\alpha}$ in inflammatory exudate at 4–48 h after UVB irradiation of human skin (summary), Suppl. 14, 21

Herrmann, W.P. and Habbig, J., immunological demonstration of multiple esterases in human eccrine sweat, 67

Hewitt, J., Frindel, E., Tubiana, M.; Guigon, M. and: study of an inhibiting factor of epidermal proliferation in plucked skin and various tumours of mice, 131

Hjorth, N., Roed-Petersen, J. and Thomsen, K., airborne contact dermatitis from Compositae oleoresins simulating photodermatitis, 613

Hodgins, M.B., Ead, R.; Cunliffe, W.J. and: a non-acne naevus. A clinical and laboratory study (summary), Suppl. 14, 18

Holst, R., eosinophilic pustular folliculitis, 661

Holt, G., Clayton, Yvonne M., Youssef, Nagwan, Wyborn, C.; Noble, W.C. and: the production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16

Holt, P.J.A., Lazarus, J. and Marks, R., the epidermis in thyroid disease, 513

Holt, Shirley, Rowell, N.R., Dodd, Janis; Hughes, P. and: thymus-dependent (T) lymphocyte deficiency in progressive systemic sclerosis, 469

Hopps, Rosamund M.; Squier, C.A. and: a study of the permeability barrier in epidermis and oral epithelium using horseradish peroxidase as a tracer *in vitro*, 123

Hopsu-Havu, V.K.; Saarni, K. and: effect of hydrocortisone butyrate on collagen synthesis (brief communication), 566

Howell, J.B.; Anderson, D.E. and: epithelioma adenoides cysticum: genetic update, 225

Howell, J.B. and Anderson, D.E., transformation of epithelioma adenoides cysticum into multiple rodent ulcers: fact or fallacy, 233

Hudson, P.M.; Black, M.M. and: atrophie blanche lesions closely resembling malignant atrophic papulosis (Degos' disease) in systemic lupus erythematosus, 649

Hudson, P.M. and Black, M.M., nodular prurigo (case report), Suppl. 14, 79

Hughes, P., Holt, Shirley, Rowell, N.R. and Dodd, Janis: thymus-dependent (T) lymphocyte deficiency in progressive systemic sclerosis, 469

Hunter, J.A.A., Zaynoun, S., Patterson, W.D., MacKie, Rona M., Cochran, A.J. and Bleehen, S.S., malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13

Hussain, S.T., Shah, S.N.A.; Hajini, H. and: sodium, potassium and phosphorus content of normal and psoriatic skin (brief communication), 674

Ichihashi, M. and Ramsay, C.A., excision repair of DNA in some photodermatoses, 13

Jackson, R., the lines of Blaschko: a review and consideration, 349

Jacobs, A.H., Cox, A.J.; Walton, R.G. and: pigmented lesions in newborn infants, 389

Jacyk, W.K., Septrin in the treatment of dermatitis cruris pustulosa et atrophicans, 71

James, M.P., Black, M.M. and Sparkes, C.G., measurement of dermal atrophy induced by topical steroids using a radiographic technique (summary), Suppl. 14, 12

James, M.P. and Gold, S.C., generalized plane xanthoma (case report), Suppl. 14, 50

James, M.P. and Naylor, P.F.D., acute widespread morphoea (case report), Suppl. 14, 34

Jankowski, E., Jankowski-Berntsen, I.; Berrens, L. and: complement component profiles in urticaria, dermatitis herpetiformis, and alopecia areata, 145

Johnson, B.E., Gould, P., Mackenzie, L.A., Frain-Bell, W.; Laksmipathi, T. and: photochemotherapy. A study of its efficacy in fifty patients suffering from psoriasis and other dermatoses (summary), Suppl. 14, 20

Joysey, V.C., McKenzie, A.W., Roger, J.H.; Williams, R.C. and: HL-A antigens in patients with guttate psoriasis, 163

Juhlin, L., Michaëlsson, G.; Ros, Ann-Mari and: a follow-up study of patients with recurrent urticaria and hypersensitivity to aspirin, benzoates and azo dyes, 19

Karvonen, J., Suhonen, R.; Hannuksela, M. and: caustic ulcers caused by cement, 547

Keczkes, K. and Farr, M., bullous dermatosis of chronic renal failure, 541

Kelly, I.; Foreman, M.I. and: the diffusion of nandrolone through hydrated human cadaver skin, 265

Kennedy, C., Sanderson, K.V. and Spry, C., painful bruising syndrome with vasculitis treated by plasmaphoresis (case report), Suppl. 14, 65

Kernohan, I.R., Fraser, N.G.; Reid, T.M.S. and: generalized warts and immune deficiency, 559

Kiiskinen, A. and Heikkinen, E., physical training and connective tissues in young mice, 525

Kirby, J. and Munro, D.D., lupoid sycosis (case report), Suppl. 14, 59

Klaber, M., Baker, H. and Warin, A., porphyria cutanea tarda (case report), Suppl. 14, 28

Klaber, M. and Pegum, J.S., xeroderma pigmentosum in brother and sister (case report), Suppl. 14, 47

Klingler, W.G., Montague, P.M; Elias, P.M. and: kinetics of *in vitro* bovine keratohyalin synthesis, 115

Kobza Black, A., Greaves, M.W., Hensby, C.N., Pazzaglia, A. and Plummer, N., increase in prostaglandin E_2 and $F_2\alpha$ in inflammatory exudate at 4–48 h after UVB irradiation of human skin (summary), Suppl. 14, 21

Kraft, D., characteristics and clinical importance of killer (K)-cells, 449

Kubba, R. and Rook, A., congenital triangular alopecia, 657

Kurban, A.K.; Al-Ahmar, H.F. and: psychological profile of patients with atopic dermatitis, 373

Laksmipathi, T., Gould, P., Johnson, B.E., MacKenzie, L.A. and Frain-Bell, W., photochemotherapy. A study of its efficacy in fifty patients suffering from psoriasis and other dermatoses (summary), Suppl. 14, 20

Lazarus, J., Marks, R.; Holt, P.J.A. and: the epidermis in thyroid disease, 513

Legget, G., Ziboh, V.A., Poitier, J., Penneys, N.S.; Lord, J.T. and: the effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins, 397

Lewis, M.G.; Rowden, G. and: Langerhans cells: involvement in the pathogenesis of mycosis fungoides, 665

Lichtig, C., Sobel, J.D., Friedman-Birnbaum, R.; Haim, S. and: histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease, 631

Liebenberg, N.v.d.W.; Findlay, G.H. and: the ultrastructure of the human epidermis following ultracentrifugation, 507

Linscheid, R.L., Goldyne, M.E.; Winkelmann, R.K. and: hypersensitivity of scleroderma cutaneous vascular smooth muscle to 5-hydroxytryptamine, 51

Lord, J.T., Ziboh, V.A., Poitier, J., Legget, G. and Penneys, N.S., the effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins, 397

Lovell, D.J., Smiljanic, R.J.; Bibel, D.J. and: effects of occlusion upon population dynamics of skin bacteria, 607

Lowe, N.J., Cudworth, A.G., Clough, S.A. and Bullen, M.F., carbohydrate metabolism in lichen planus, 9

Lowe, N.J., Cudworth, A.G. and Woodrow, J.C., HL-A antigens in lichen planus, 169

McCallum, D.I. and Gray, W.M., metastatic Crohn's disease, 551

MacDonald, A., Nicholson, D. and Williams, R., late onset erythropoietic uroporphyria (case report), Suppl. 14, 30

MacDonald, D.M. and Greaves, M.W., Cornelia de Lange syndrome (case report), Suppl. 14, 37

MacDonald, D.M., Morrison, J.G.L. and Greaves, M., cutaneous ectopic schistosomiasis (case report) Suppl. 14, 57

MacDonald, D.M. and Nicholson, D.C., erythropoietic protoporphyria: hepatic implications, 157

McKenzie, A.W., Roger, J.H., Joysey, V.C.; Williams, R.C. and: HL-A antigens in patients with guttate psoriasis, 163

McKenzie, A.W., Stubbing, D.G. and Elvy, B.L., prurigo nodularis and gluten enteropathy, 89

Mackenzie, L.A., Gould, P., Johnson, B.E., Frain-Bell, W.; Laksmipathi, T. and: photochemotherapy. A study of its efficacy in fifty patients suffering from psoriasis and other dermatoses (summary), Suppl. 14, 20

MacKie, Rona M., Zaynoun, S., Patterson, W.D., Cochran, A.J., Bleehen, S.S.; Hunter, J.A.A. and: malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13

MacSween, R.N.M., Thomson, J.; Cochran, Rebecca E.I. and: an auto-antibody profile in alopecia totalis and diffuse alopecia, 61

Magnus, I.A., Woodcock, A. and: the sunburn cell in mouse skin: preliminary quantitative studies on its production, 459

Mahrle, G. and Orfanos, C.E., localization and activity of tissue bound cyclic nucleotide phosphodiesterase in normal and lack of changes in psoriatic human skin, 591

Maibach, H.I., Mandel, A.; Aly, R. and: bacterial flora in psoriasis, 603

Maibach, H.I.; Wachs, G.N. and: co-operative double-blind trial of an antibiotic/corticoid combination in impetiginized atopic dermatitis, 323

Mäkelä, P.H., Valtonen, V.V., Salo, O.P.; Valtonen, M.V. and: the effect of long term tetracycline treatment for acne vulgaris on the occurrence of R factors in the intestinal flora of man, 311

Mali, J.W.H., Bergers, A.M.G., van den Hurk, J.J.M.A., Mier, P.D. and van de Staak, W.J.B.M., a lysosomal storage disorder of the epidermis characterized by a deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627

Mandel, A., Maibach, H.I.; Aly, R. and: bacterial flora in psoriasis, 603

Marks, R.; Davies, M. and: studies on the effect of salicylic acid on normal skin, 187

Marks, R., Greaves, M.W., Danks, D. and Plummer, V., trimethylaminuria or fish odour syndrome in a child (summary), Suppl. 14, 11

Marks, R., Lazarus, J.; Holt, P.J.A. and: the epidermis in thyroid disease, 513

Marsh, P.D., *in vitro* studies of antagonism among human skin bacteria (thesis summary), 571

Marten, R.H.; Byrne, J.P.H. and: lichen myxoedematosus (case report), Suppl. 14, 52

Matthews, C.N., Harman, R.R.M. and Warin, R.P., multiple basal cell carcinomata occurring in areas previously exposed to excessive amounts of diagnostic X-rays in 3 patients (case), 211

Meynadier, J., Clot, J., Charmasson, E., Dardenne, M., Brochier, J.; Guilhou, J.J. and: immunological aspects of psoriasis. II. Dissociated impairment of thymus-dependent lymphocytes, 295

Michäelsson, G., Juhlin, L.; Ros, Ann-Mari and: a follow-up study of patients with recurrent urticaria urticaria and hypersensitivity to aspirin, benzoates and azo dyes, 19

Mier, P.D., Bergers, A.M.G., van den Hurk, J.J.M.A., van de Staak, W.J.B.M., Mali, J.W.H. and: a lysosomal storage disorder of the epidermis characterized by a deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627

Mier, P.D. and van den Hurk, J.J.M.A., lysosomal hydrolases of the epidermis. 6. Changes in disease, 271

Mier, P.D., van den Hurk, J.J.M.A., Roelfzema, H.; Volden, G. and: first demonstration of cathepsin A in epidermis (brief communication), 675

Mieras, C., Ferrando, J., Tomás, J.M., Peyrí, J.; Piñol-Aguadé, J. and: necropsy and ultrastructural findings in histiocytic medullary reticulosis, 35

Milyani, Rajaa M.M., studies on interactions of human skin micro-organisms on solid surfaces (thesis summary), 571

Montague, P.M., Klingler, W.G.; Elias, P.M. and: kinetics of *in vitro* bovine keratohyalin synthesis, 115

Morgan, J.K.; Musgrove, K. and: infantile eczema: a long-term follow-up study, 365

Morrison, J.G.L., sarcoidosis in a child, presenting as an erythroderma with keratotic spines and palmar pits, 93

Morrison, J.G.L., Greaves, M.; MacDonald, D.M. and: cutaneous ectopic schistosomiasis (case report), Suppl. 14, 57

Moynahan, E.J.; Sparrow, G.P. and: localized: pemphigoid (case report), Suppl. 14, 26

Munro, D.D., bathing trunk naevus with neoplastic transformation (case report), Suppl. 14, 77

Munro, D.D.; Kirby, J. and: lupoid sycosis (case report), Suppl. 14, 59

Musgrove, K. and Morgan, J.K., infantile eczema: a long-term follow-up study, 365

Naylor, P.F.D.; James, M.P. and: acute widespread morphoea (case report), Suppl. 14, 34

Newbold, P.C.H., β-carotene in the treatment of discoid lupus erythematosus, 100

Nicholson, D., Williams, R.; MacDonald, A. and: late onset erythropoietic uroporphyria (case report), Suppl. 14, 30

Nicholson, D.C.; Macdonald, D.M. and: erythropoietic protoporphyria: hepatic implications, 157

Noble, W.C., Clayton, Yvonne M., Youssef, Nagwan, Holt, G. and Wyborn, C., the production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16

O'Brien, J.P., is actinic (solar) damage the provoking cause of 'post-inflammatory elastolysis and cutis laxa (PECL)'? (letter), 105

Ofuji, S.; Tagami, H. and: leukotactic properties of soluble substances in psoriasis scale, 1

Orfanos, C.E.; Mahrle, G. and: localization and activity of tissue bound cyclic nucleotide phosphodiesterase in normal and lack of changes in psoriatic human skin, 591

Orfanos, C.E. and Runne, U., systemic use of a new retinoid with and without local dithranol treatment in generalized psoriasis, 101

Ortonne, J.P., Bettuel, H., Thivolet, J.; Retornaz, G. and: HL-A antigens and vitiligo, 173

Osterman, P.O., paroxysmal itching in multiple sclerosis, 555

Parish, W.E., Welbourn, Esther, and Champion, R.H., hypersensitivity to bacteria in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci, 285. III. Arthus-like responses to bacterial antigens without specific antibody, 379. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens, 493

Parving, H.-H., Worm, Anne-Marie and Rossing, N., plasma volume, intravascular albumin and its transcapillary escape rate in patients with extensive skin disease, 519

Paslin, D., causation of psoriatic epithelial hyperproliferation (letter), 106

Patterson, W.D., Zaynoun, S., MacKie, Rona M., Cochran, A.J., Bleehen, S.S.; Hunter, J.A.A. and: malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type (summary), Suppl. 14, 13

Pazzaglia, A., Greaves, M.W., Hensby, C.N., Plummer, N.; Kobza Black, A. and: increase in prostaglandin E_2 and $F_{2\alpha}$ in inflammatory exudate at 4–48 g after UVB irradiation of human skin (summary), Suppl. 14, 21

Peachey, R.D.G., molluscum contagiosum with giant lesions (case), 213

Peachey, R.D.G., Pye, R.J. and Harman, R.R.M., the treatment of psoriatic nail dystrophy with intradermal steroid injections, 75

Pegum, J.S.; Klaber, M. and: xeroderma pigmentosum in brother and sister (case report), Suppl. 14, 47

Peiris, S. and Williams, D.I., acnitis (case report), Suppl. 14, 58

Penneys, N.S., Ziboh, V.A., Poitier, J., Legget, G., Lord, J.T. and: the effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins, 397

Perera, W.D.H., Thackray, P., Williams, M., Forster, R.A., Williams, S.M.; Cunliffe, W.J. and: pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153

Perry, H.O., Rogers, R.S.III; Person, J.R. and: localized pemphigoid, 531

Person, J.R. and Rogers, R.S.III, bullous pemphigoid and psoriasis: does subclinical bullous pemphigoid exist?, 535.

Person, J.R., Rogers, R.S.III and Perry, H.O., localized pemphigoid, 531

Peyrí, J., Ferrando, J., Tomás, J.M., Mieras, C.; Piñol-Aguadé, J. and: necropsy and ultrastructural findings in histiocytic medullary reticulosis, 35

Philpot, Christine M., the differentiation of dermatophytes by biochemical and serological methods (thesis summary), 453

Picton, W., Devitt, H. and Forgie, M.A., practical applications of the quantimet 720, an image analysing computer, in the field of investigative dermatology, 341

Pinkus, H.; Bedi, T.R. and: histopathological spectrum of erythema multiforme, 243

Piñol-Aguadé, J., Ferrando, J., Tomás, J.M., Meiras, C. and Peyrí, J., necropsy and ultrastructural findings in histiocytic medullary reticulosis, 35

Pirozzi, D.J. and Rosenthal, A., disseminated superficial actinic porokeratosis, 429

Pitcher, D.G., taxonomy of aerobic cutaneous diphtheroids (summary), Suppl. 14, 17

Plummer, N., Greaves, M.W., Hensby, C.N., Pazzaglia, A.; Kobza Black, A. and: increase in prostaglandin E_2 and $F_{2\alpha}$ in inflammatory exudate at 4–48 h after UVB irradiation of human skin (summary), Suppl. 14, 21

Plummer, V., Greaves, M.W., Danks, D.; Marks, R. and: trimethylaminuria or fish odour syndrome in a child (summary), Suppl. 14, 11

Poitier, J., Ziboh, V.A., Legget, G., Penneys, N.S.; Lord, J.T. and: the effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins, 397

Powell, M.E.A., Rhodes, E.L., Edwards, C.O.; Gange, R.W. and: pemphigus by rifampicin, 445

Prottey, C.; Hartop, P.J. and: changes in transepidermal water loss and the composition of epidermal lecithin after applications of pure fatty acid triglycerides to the skin of essential fatty acid-deficient rats, 255

Pye, R.J., Brookes, D.B.; Burton, J.L. and: metal corrosion by chloride in sweat, 417

Pye, R.J. and Burton, J.L., multiple cutaneous malignancies in leukaemia (case), 210

Pye, R.J., Burton, J.L. and Harris, J.I., effect of 1% cyproterone acetate in Cetomacrogol cream BPC (Formula A) on sebum excretion rate in patients with acne, 427

Pye, R.J., Harman, R.R.M.; Peachey, R.D.G. and: the treatment of psoriatic nail dystrophy with intradermal steroid injections, 75

Raab, W.P., dithranol (anthralin) versus triacetoxyanthracene: investigations *in vitro*, 193

Ramsay, C.A.; Ichihashi, M. and: excision repair of DNA in some photodermatoses, 13

Rasmussen, J.E., erythema multiforme in children: response to treatment with systemic corticosteroids, 181

Reid, T.M.S., Fraser, N.G. and Kernohan, I.R., generalized warts and immune deficiency, 559

Rendall, J.R.S. and Robinson, T., lichen aureus (case report), Suppl. 14, 45

Retornaz, G., Betuel, H., Ortonne, J.P. and Thivolet, J., HL-A antigens and vitiligo, 173

Rhodes, E.L., fibrinolytic agents in the treatment of necrobiosis lipoidica (brief communication), 673

Rhodes, E.L., relapsing polychondritis (case report), Suppl. 14, 81

Rhodes, E.L., Edwards, C.O., Powell, M.E.A.; Gange, R.W. and: pemphigus induced by rifampicin, 445

Roberts, C.D., the role of bacteria in acne vulgaris (thesis summary), 453

Robinson, T.; Rendall, J.R.S. and: lichen aureus (case report), Suppl. 14, 45

Robinson, T.; Taylor, D.R. and: piebaldism (case report), Suppl. 14, 43

Roed-Petersen, J., Thomsen, K.; Hjorth, N. and: airborne contact dermatitis from Compositae oleoresins simulating photodermatitis, 613

Roelfzema, H., van den Hurk, J.J.M.A., Mier, P.D.; Volden, G. and: first demonstration of cathepsin A in epidermis (brief communication), 675

Roger, J.H., McKenzie, A.W., Joysey, V.C.; Williams, R.C. and: HL-A antigens in patients with guttate psoriasis, 163

Rogers, R.S.III, Perry, H.O.; Person, J.R. and: localized pemphigoid, 531

Rogers, R.S.III; Person, J.R. and: bullous pemphigoid and psoriasis: does subclinical bullous pemphigoid exist?, 535

Rook, A., the clinical importance of 'weathering' in human hair, 111

Rook, A.; Kubba, R. and: congenital triangular alopecia, 657

Ros, Ann-Mari, Juhlin, L. and Michaëlsson, G., a follow-up study of patients with recurrent urticaria and hypersensitivity to aspirin, benzoates and azo dyes, 19

Rosenthal, A.; Pirozzi, D.J. and: disseminated superficial actinic porokeratosis, 429

Rossing, N., Worm, Anne-Marie; Parving, H.-H. and: plasma volume, intravascular albumin and its transcapillary escape rate in patients with extensive skin disease, 519

Rowden, G. and Lewis, M.G., Langerhans cells: involvement in the pathogenesis of mycosis fungoides, 665

Rowell, N.R., digital ischaemia due to vascular anomalies (summary), Suppl. 14, 20

Rowell, N.R., the prognosis of systemic sclerosis, 57

Rowell, N.R., Holt, Shirley, Dodd, Janis; Hughes, P. and: thymus-dependent (T) lymphocyte deficiency in progressive systemic sclerosis, 469

Rowell, N.R.; Tan, S.G. and: pemphigus-like syndrome induced by D-penicillamine, 99

Runne, U.; Orfanos, C.E. and: systemic use of a new retinoid with and without local dithranol treatment in generalized psoriasis, 101

Saarni, K. and Hopsu-Havu, V.K., effect of hydrocortisone butyrate on collagen synthesis (brief communication), 566

Salo, O.P., Valtonen, V.V., Mäkelä, P.H.; Valtonen, M.V. and: the effect of long term tetracycline treatment for acne vulgaris on the occurrence of R factors in the intestinal flora of man, 311

Samman, P.D., Sharvill, D.E.; Staughton, R.C.D. and: Sézary syndrome (case report), Suppl. 14, 75

Sanderson, K.V.; Smith, N. and: lipoid proteinosis (case report), Suppl. 14, 48

Sanderson, K.V., Spry, C.; Kennedy, C. and: painful bruising syndrome with vasculitis treated by plasmaphoresis (case report), Suppl. 14, 65

Sarkany, I.; Barnes, H.M. and: adverse skin reaction from vitamin K1, 653

Sarkany, I.; Burns, D.A. and: generalized morphoea with bullous lesions and psoriasis (case report), Suppl. 14, 33

Sarkany, I.; Burns, D.A. and: management of stasis ulcers by pinch graft (case report), Suppl. 14, 82

Scherer, R. and Braun-Falco, O., alternative pathway complement activation: a possible mechanism inducing skin lesions in benign gonococcal sepsis, 303

Schmidt, H.; Thomsen, K. and: PUVA-induced bullous pemphigoid (brief communication), 568

Sehgal, V.N., Ciclopirox: a new topical pyrodonium antimycotic agent, 83

Senior, Jennifer, Hartley, Lisa; Bleehen, S.S. and: lentigo maligna melanoma: an ultrastructural study (summary), Suppl. 14, 14

Seville, R.H., relapse rate of psoriasis worsened by adding steroids to a dithranol regime, 643

Shah, S.N.A., Hussain, S.T.; Hajini, H. and: sodium, potassium and phosphorus content of normal and psoriatic skin (brief communication), 674

Sharvill, D.E., Samman, P.D.; Staughton, R.C.D. and: Sézary syndrome (case report), Suppl. 14, 75

Shelley, W.B. and Goldwein, M.I., high dose dapsone toxicity, 79

Shoeib, S., Aziz, A.A., Soliman, M., Tadros, S.S.; El Zawahry, M. and: clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules for the treatment of skin infections, 177

Skeete, M. and Greaves, M.W., juvenile bullous pemphigoid (case report), Suppl. 14, 23

Smiljanic, R.J., Lovell, D.J.; Bibel, D.J. and: effects of occlusion upon population dynamics of skin bacteria, 607

Smith, N. and Sanderson, K.V., lipoid proteinosis (case report), Suppl. 14, 48

Sobel, J.D., Friedman-Birnbaum, R., Lichtig, C.; Haim, S. and: histological and direct immunofluorescence study of cutaneous hyperreactivity in Behçet's disease, 631

Soliman, M., Aziz, A.A., Shoeib, S., Tadros, S.S.; El Zawahry, M. and: clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules for the treatment of skin infections, 177

Sparkes, C.G., Black, M.M.; James, M.P. and: measurement of dermal atrophy induced by topical steroids using a radiographic technique (summary), Suppl. 14, 12

Sparrow, G.P. and Moynahan, E.J., localized pemphigoid (case report), Suppl. 14, 26

Spry, C., Sanderson, K.V.; Kennedy, C. and: painful bruising syndrome with vasculitis treated by plasmaphoresis (case report), Suppl. 14, 65

Squier, C.A. and Hopps, Rosamund M., a study of the permeability barrier in epidermis and oral epithelium using horseradish peroxidase as a tracer *in vitro*, 123

Stankler, L. and Walker, F., periodic-acid-Schiff staining for glycogen in clinically normal psoriatic and non-psoriatic skin, 599

Staughton, R.C.D. and Copeman, P.W.M., chronic intravascular coagulopathy with ?pyoderma gangrenosum (case report), Suppl. 14, 70

Staughton, R.C.D., Samman, P.D. and Sharvill, D.E., Sézary syndrome (case report), Suppl. 14, 75

Stephens, A., Comaish, J.S., Hassanyeh, F.; Goolamali, S.K. and: familial Behçet's syndrome, 637

Stewart, W.M., pathology of lichen striatus (summary), Suppl. 14, 18

Strong, Alexandra M.M., Thomson, J., Fleming, K.A.; Cochran, Rebecca E.I. and: histology simulating reticulosis in secondary syphilis, 251

Stubbing, D.G., Elvy, B.L.; McKenzie, A.W. and: prurigo nodularis and gluten enteropathy, 89

Sugathan, P., mudi-chood on the pinnae, 197

Suhonen, R., Karvonen, J.; Hannuksela, M. and: caustic ulcers caused by cement, 547

Tadros, S.S., Aziz, A.A., Soliman, M., Shoeib, S.; El Zawahry, M. and: clinical and bacteriological evaluation of a twice daily regimen of epicillin capsules for the treatment of skin infections, 177

Tagami, H. and Ofuji, S., leukotactic properties of soluble substances in psoriasis scale, 1

Tan, S.G., acne vulgaris: a laboratory and clinical study with special reference to microbial factors in pathogenesis (thesis summary), 571

Tan, S.G. and Rowell, N.R., pemphigus-like syndrome induced by D-penicillamine, 99

Tan, Tulip: the effect of collagenase on surface markers of human peripheral lymphocytes, 361

Taylor, D.R. and Robinson, T., piebaldism (case report), Suppl. 14, 43

Thackray, P., Perera, W.D.H., Williams, M., Forster, R.A., Williams, S.M.; Cunliffe, W.J. and: pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153

Thivolet, J., Betuel, H., Ortonne, J.P.; Retornaz, G. and: HL-A antigens and vitiligo, 173

Thomsen, K., Roed-Petersen, J.; Hjorth, N. and: airborne contact dermatitis from Compositae oleoresins simulating photodermatitis, 613

Thomsen, K. and Schmidt, H., PUVA-induced bullous pemphigoid (brief communication), 568

Thomson, J., Fleming, K.A., Strong, Alexandra M.M.; Cochran, Rebecca E.I. and: histology simulating reticulosis in secondary syphilis, 251

Thomson, J., MacSween, R.N.M.; Cochran, Rebecca E.I. and: an auto-antibody profile in alopecia totalis and diffuse alopecia, 61

Tomás, J.M., Ferrando, J., Mieras, C., Peyrí, J.; Pinol-Aguadé, J. and: necropsy and ultrastructural findings in histiocytic medullary reticulosis, 35

Tsuji, T., subcutaneous fat necrosis of the newborn: light and electron microscopic studies, 407

Tubiana, M., Frindel, E., Hewitt, J.; Guigon, M. and: study of an inhibiting factor of epidermal proliferation in plucked skin and various tumours of mice, 131

Umbert, P. and Winkelmann, R.K., granuloma annulare: direct immunofluorescence study, 487

Umbert, P., Belcher, R.W. and Winkelmann, R.K., lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare, 481

Umbert, P., Belcher, R.W., Winkelmann, R.K. and: macrophage inhibitor factor (MIF) in cutaneous lymphoproliferative diseases, 475

Valdimarsson, H., Feiwel, M.; Clayton, R. and: pyoderma gangrenosum with cellular immunity deficit treated with plasmaphoresis and leukocyte transfusion (case report), Suppl. 14, 67

Valtonen, M.V., Valtonen, V.V., Salo, O.P. and Makela, P.H., the effect of long term tetracycline treatment for acne vulgaris on the occurrence of R factors in the intestinal flora of man, 311

van de Staak, W.J.B.M., Bergers, A.M.G., van den Hurk, J.J.M.A., Mier, P.D.; Mali, J.W.H. and: a lysosomal storage disorder of the epidermis characterized by a deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627

van den Hurk, J.J.M.A., Bergers, A.M.G., Mier, P.D., van de Staak, W.J.B.M.; Mali, J.W.H. and: a lysosomal storage disorder of the epidermis characterized by a deficiency of α-mannosidase and an accumulation of mannose-rich materials, 627

van den Hurk, J.J.M.A.; Mier, P.D. and: lysosomal hydrolases of the epidermis. 6. Changes in disease, 271

van den Hurk, J.J.M.A., Mier, P.D., Roelfzema, H.; Volden, G. and: first demonstration of cathepsin A in epidermis (brief communication), 675

Varga, J., Elias, P.; Fritsch, P. and: the fate of staphylococcal exfoliatin in newborn and adult mice, 275

Verbov, J., muscle herniation of the lower legs (brief communication), 329

Volden, G., van den Hurk, J.J.M.A., Mier, P.D. and Roelfzema, H., first demonstration of cathepsin A in epidermis (brief communication), 675

Vollum, D.I., erythroderma and vasculitis (case report), Suppl. 14, 74

Vollum, D.I., multiple keratoacanthomas (case report), Suppl. 14, 78

Vollum, D.I., pyoderma gangrenosum, hypoplastic anaemia, tabes dorsalis (case report), Suppl. 14, 69

Vollum, D.I., sarcoidosis (case report), Suppl. 14, 56

Wachs, G.N. and Maibach, H.I., co-operative double-blind trial of an antibiotic/corticoid combination in impetiginized atopic dermatitis, 323

Walker, F.; Stankler, L. and: periodic-acid-Schiff staining for glycogen in clinically normal psoriatic and non-psoriatic skin, 599

Wall, M., Cross, K.W., Fairburn, E.A.; Bowker N.C. and: sociological implications of an epidemiological study of eczema in the City of Birmingham, 137

Walton, R.G., Jacobs, A.H. and Cox, A.J., pigmented lesions in newborn infants, 389

Ward, Joanna M., Corbett, Margaret F. and Hanna, M.J., a double-blind trial of clomocycline in the treatment of persistent palmoplantar pustulosis, 317

Warin, A.P., Baker, H.; Klaber, M. and: porphyria cutanea tarda (case report), Suppl. 14, 28

Warin, A.P. and Wilson Jones, E., cutaneous tuberculosis of the nose with unusual clinical and histological features leading to delay in diagnosis (summary), Suppl. 14, 15

Warin, R.P., Harman, R.R.M.; Matthews, C.N. and: multiple basal cell carcinomata occurring in areas previously exposed to excessive amounts of diagnostic X-rays in 3 patients (case), 211

Weber, G., effects and side-effects of 8-methoxypsoralen-black light therapy in more than 1500 patients (summary), Suppl. 14, 21

Welbourn, Esther, Champion, R.H.; Parish, W.E. and: hypersensitivity to bacteria in eczema. II. Titre and immunoglobulin class of antibodies to staphylococci and micrococci, 285. III. Arthus-like responses to bacterial antigens without specific antibody, 379. IV. Cytotoxic effect of antibacterial antibody on skin cells acquiring bacterial antigens, 493

WELLS, G.C.; BENTLEY-PHILLIPS, C.B. and: transient acantholytic dermatosis (case report), Suppl. 14, 25

WELLS, G.C.; CLARKE, JACQUELINE and: livedo reticularis with thrombocythaemia in association with polycythaemia rubra vera (case report), Suppl. 14, 63

WELLS, R.S.; ATHERTON, D.J. and: ?sclerodema of Buschke ?Eosinophilic fasciitis (case report), Suppl 14, 36

WELLS, R.S.; BESSER, F. and: the tricho-rhino-phalangeal syndrome (case report), Suppl. 14, 39

WHIMSTER, I.W., nerve supply as a stimulator of the growth of tissues, including skin (summary), Suppl. 14, 9

WILLIAMS, D.I.; BYRNE, J.P.H. and: bisalbuminaemia (case report), Suppl. 14, 54

WILLIAMS, D.I.; BYRNE, J.P.H. and: case for diagnosis (case report), Suppl. 14, 83

WILLIAMS, D.I.; PEIRIS, S. and: acnitis (case report), Suppl. 14, 58

WILLIAMS, M., PERERA, W.D.H., THACKRAY, P., FORSTER, R.A., WILLIAMS, S.M.; CUNLIFFE, W.J. and: pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153

WILLIAMS, R., NICHOLSON, D.; MACDONALD, A. and: late onset erythropoietic uroporphyria (case report), Suppl. 14, 30

WILLIAMS, R.C., MCKENZIE, A.W., ROGER, J.H. and JOYSEY, V.C., HL-A antigens in patients with guttate psoriasis, 163

WILLIAMS, S.M., PERERA, W.D.H., THACKRAY, P., WILLIAMS, M., FORSTER, R.A.; CUNLIFFE, W.J. and: pilo-sebaceous duct physiology. III. Observations on the number and size of pilo-sebaceous ducts in acne vulgaris, 153

WILSON JONES, E., COWEN, T.; EADY, R.A.J. and: pseudopyogenic granuloma: the histopathogenesis in the light of ultrastructural studies (summary), Suppl. 14, 13

WILSON JONES, E.; WARIN, A.P. and: cutaneous tuberculosis of the nose with unusual clinical and histological features leading to delay in diagnosis (summary), Suppl. 14, 14

WINKELMANN, R.K., BELCHER, R.W.; UMBERT, P. and: lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare, 481

WINKELMANN, R.K., BELCHER, R.W.; UMBERT, P. and: macrophage inhibitor factor (MIF) in cutaneous lymphoproliferative diseases, 475

WINKELMANN, R.K., GOLDYNE, M.E. and LINSCHEID, R.L., hypersensitivity of scleroderma cutaneous vascular smooth muscle to 5-hydroxytryptamine, 51

WINKELMANN, R.K.; UMBERT, P. and: granuloma annulare: direct immunofluorescence study, 487

WOODCOCK, A. and MAGNUS, I.A., the sunburn cell in mouse skin: preliminary quantitative studies on its production, 459

WOODROW, J.C., CUDWORTH, A.G.; LOWE, N.J. and: HL-A antigens in lichen planus, 169

WORM, ANNE-MARIE, ROSSING, N.; PARVING, H.-H. and: plasma volume, intravascular albumin and its transcapillary escape rate in patients with extensive skin disease, 519

WUEPPER, K.D., ERICKSON, K.L.; DIMOND, R.L. and: the role of divalent cations in epidermolysis, 25

WYBORN, C., CLAYTON, YVONNE M., YOUSSEF, NAGWAN, HOLT, G.; NOBLE, W.C. and: the production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16

YARDLEY, H.J. and GOLDSTEIN, D.J., changes in dry weight and projected area of human epidermal cells undergoing keratinization as determined by scanning interference microscopy, 621

YOUSSEF, NAGWAN, CLAYTON, YVONNE M., HOLT, G., WYBORN, C.; NOBLE, W.C. and: the production of antibiotics by *Trichophyton* and *Epidermophyton* species (summary), Suppl. 14, 16

ZAYNOUN, S., PATTERSON, W.D., MACKIE, RONA M., COCHRAN, A.J., BLEEHEN, S.S.; HUNTER, J.A.A. and: malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type, Suppl. 14, 13

ZIBOH, V.A., POITIER, J., LEGGET, G., PENNEYS, N.S.; LORD, J.T. and: the effects of photosensitizers and ultraviolet irradiation on the biosynthesis and metabolism of prostaglandins, 397

ZINA, A., ZINA, G.; BERNENGO, M.G. and: leucosis and skin: acute lymphoid immunoblastic leukaemia, 45

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr R. H. Champion, Aldenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldly names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	seconds(s)	s	gravitational acceleration	*g*
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors, all names should be given when cited for the first time and thereafter only the first name adding *et al.*, e.g. Blackett *et al.* (1952); for four or more authors, the first name followed by *et al.* should be used on all occasions. If several papers by the same author and from the same year are cited, a, b, c, etc., should be put after the year of publication, e.g. Blackett *et al.* (1952a) or Blackett *et al.* (1952a, b). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s); (2) year of publication in parentheses; (3) title of paper; (4) title of Journal in full; (5) volume; (6) number of the first page of the article. *Thus*: Cunliffe, W.J. & Shuster, S. (1969) The rate of sebum excretion in man. *British Journal of Dermatology*, 81, 697. References to the books and monographs should include: (1) author(s) or editor(s); (2) year of publication: (3) title; (4) edition, volume etc.; (5) page referred to; (6) publisher; (7) place. *Thus*: Beare, J.M. & Wilson Jones, E. (1972) Necrobiotic disorders. In: *Textbook of Dermatology* (Ed. by A.J.Rook, D.S.Wilkinson and F.J.Ebling), 2nd edn, Vol. 2, p. 1066, Blackwell Scientific Publications, Oxford.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

British Journal of Dermatology, Volume 95, Number 6, December 1976

CONTENTS

Page

Clinical and Laboratory Investigations

The Cell Cycle in Psoriasis: a Reappraisal 577
SEYMOUR GELFANT
Localization and Activity of Tissue Bound Cyclic Nucleotide Phosphodiesterase in Normal and Lack of Changes in Psoriatic Human Skin 591
G.MAHRLE AND C.E.ORFANOS
Periodic Acid-Schiff (PAS) Staining for Glycogen in Clinically Normal Psoriatic and Non-psoriatic Skin 599
LESLIE STANKLER AND FREDERICK WALKER
Bacterial Flora in Psoriasis 603
RAZA ALY, HOWARD I.MAIBACH AND ADRIAN MANDEL
Effects of Occlusion upon Population Dynamics of Skin Bacteria 607
DAVID J.BIBEL, DAVID J.LOVELL AND ROKO J.SMILJANIC
Airborne Contact Dermatitis from Compositae Oleoresins Simulating Photodermatitis 613
NIELS HJORTH, JYTTE ROED-PETERSEN AND KRISTIAN THOMSEN
Changes in Dry Weight and Projected Area of Human Epidermal Cells Undergoing Keratinization as Determined by Scanning Interference Microscopy 621
H.J.YARDLEY AND D.J.GOLDSTEIN
A Lysosomal Storage Disorder of the Epidermis Characterized by a Deficiency of α-Mannosidase and an Accumulation of Mannose-rich Materials 627
J.W.H.MALI, A.M.G.BERGERS, JOSÉ J.M.A. VAN DEN HURK, P.D.MIER AND W.J.B.M. VAN DE STAAK
Histological and Direct Immunofluorescence Study of Cutaneous Hyperreactivity in Behçet's Disease 631
S.HAIM, J.D.SOBEL, R.FRIEDMAN-BIRNBAUM AND C.LICHTIG
Familial Behçet's Syndrome 637
S.K.GOOLAMALI, J.S.COMAISH, F.HASSANYEH AND A.STEPHENS

Pharmacology and Treatment

Relapse Rate of Psoriasis Worsened by Adding Steroids to a Dithranol Regime 643
R.H.SEVILLE

Case Reports

Epidermal Naevus Syndrome 647
M.K.ATTIA AND A.M.ABDEL-AZIZ
Atrophie Blanche Lesions Closely Resembling Malignant Atrophic Papulosis (Degos' Disease) in Systemic Lupus Erythematosus 649
MARTIN M.BLACK AND PETER M.HUDSON
Adverse Skin Reaction from Vitamin K1 653
H.M.BARNES AND I.SARKANY
Congenital Triangular Alopecia 657
RAJ KUBBA AND ARTHUR ROOK
Eosinophilic Pustular Folliculitis. Report of a European Case 661
ROLF HOLST

Comment Article

Langerhans Cells: Involvement in the Pathogenesis of Mycosis Fungoides 665
G.ROWDEN AND M.G.LEWIS

Brief Communications

Fibrinolytic Agents in the Treatment of Necrobiosis Lipoidica 673
E.L.RHODES
Sodium, Potassium and Phosphorus Content of Normal and Psoriatic Skin 674
G.H.HAJINI, S.T.HUSSAIN AND S.N.A.SHAH
First Demonstration of Cathepsin A in Epidermis 675
G.VOLDEN, JOSÉ J.M.A. VAN DEN HURK, P.D.MIER AND H.ROELFZEMA

Obituaries

Dr G.B.Dowling 677
Dr J.Savatard, Dr W.B.Reed, Dr M.Farb and Dr W.W.Gunther 681
Correspondence 682
Book Reviews 682
News and Notices 686
Index to Volume 95 691

British Association of Dermatologists

OFFICERS AND COMMITTEE 1975–6

President **DR D.I.WILLIAMS**

Immediate Past President DR A.J.ROOK
Treasurer DR O.L.S.SCOTT
Vice-President DR R.P.WARIN
Editor DR R.H.CHAMPION
Secretary DR K.V.SANDERSON

Ordinary Members of the Committee

DR D.BURROWS
DR ETAIN CRONIN
DR K.D.CROW
DR W.FRAIN-BELL
DR M.GARRETTS
PROF. M.W.GREAVES
DR P.HALL-SMITH
DR A.W.McKENZIE
DR D.D.MUNRO

Willan Librarian DR A.J.ROOK

British Journal of Dermatology is published monthly. Subscription price £21.00 (overseas £25.00; U.S.A. and Canada $75.00) per annum, post free. Subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL, England.

Printed and bound in Great Britain at the Alden Press, Oxford

British Journal of Dermatology (1976) **95**, Supplement **14**, 1.

Foreword

As in previous years this supplement contains summaries of the papers and details of the cases to be presented at the fifty-sixth annual meeting of the British Association of Dermatologists.

Those attending the Meeting are asked to bring the Supplement with them.

Once again we are extremely grateful to Glaxo Laboratories Limited for their financial support.

R.H.CHAMPION

DAVID I. WILLIAMS

PRESIDENT OF THE BRITISH ASSOCIATION OF DERMATOLOGISTS, 1975–6.

KING'S COLLEGE HOSPITAL, LONDON.

British Journal of Dermatology (1976) **95**, Supplement **14**, 5.

British Association of Dermatologists
Fifty-Sixth Annual Meeting, 1976
London

PRESIDENT: DR DAVID I.WILLIAMS

PROGRAMME OF MEETING

Thursday 8 July

Royal College of Physicians, St Andrew's Place, Regent's Park, London

09.00	Registration	
09.30	Annual General Meeting (Members only)	
10.30	**Coffee**	
10.45	I.W.Whimster	Nerve supply as a stimulator of the growth of tissues, including skin
11.05	O.Braun-Falco and G.Burg	Enzyme cytochemical and immunocytological characterization of cutaneous lymphomas
11.25	Christine Harrington	Mycosis fungoides with leukaemic transformation
11.45	Robin Marks, M.W.Greaves, D.Danks and V.Plummer	Trimethylaminuria or fish odour syndrome in a child
12.05	M.P.James, M.M.Black and C.G.Sparkes	Measurement of dermal atrophy induced by topical steroids using a radiographic technique
12.25	R.A.J.Eady, T.Cowen and E.Wilson Jones	Pseudopyogenic granuloma. The histopathogenesis in the light of ultrastructural studies

12.45	**Lunch**	
14.00	J.A.A.Hunter, S.Zaynoun, W.D.Patterson, R.M.MacKie, A.J.Cochran and S.S.Bleehen	Malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type
14.20	S.S.Bleehen, Lisa Hartley and Jennifer Senior	Lentigo-maligna melanoma: an ultrastructural study
14.40	A.P.Warin and E.Wilson Jones	Cutaneous tuberculosis of the nose with unusual clinical and histological features leading to delay in diagnosis
15.00	W.C.Noble, Yvonne M.Clayton, Nagwan Youssef, G.Holt and C.Wyborn	The production of antibiotics by *Trichophyton* and *Epidermophyton* species
15.20	**Tea**	
15.50	D.G.Pitcher	Taxonomy of aerobic cutaneous diphtheroids
16.10	W.J.Cunliffe, M.B.Hodgkins and R.Ead	A non-acne naevus—A clinical and laboratory study
16.30	W.M.Stewart	Pathology of lichen striatus
18.30	President's Reception at the Royal College of Obstetricians and Gynaecologists	

Friday 9 July

Royal College of Physicians, St Andrew's Place, Regent's Park, London

09.30	Rudolf L.Baer	Cutaneous immunology—then and now. Reflections on the period 1876–1976.
10.00	J.A.Carruthers and M.M.Black	Further studies in herpes gestationis
10.20	Neville R.Rowell	Digital ischaemia due to vascular anomalies
10.40	**Coffee**	

11.10	T.Laksmipathi, P.Gould, B.E.Johnson, L.A.Mackenzie and W.Frain-Bell	Photochemotherapy: a study of its efficacy in fifty patients suffering from psoriasis and other dermatoses
11.30	Gerhard Weber	Effects and side effects of 8-methoxy-psoralen-blacklight therapy in more than 1500 patients
11.50	A.Kobza Black, M.W.Greaves, C.N.Hensby, A.Pazzaglia and N.Plummer	Increase in prostaglandin E_2 and $F_{2\alpha}$ in inflammatory exudate at 4–48 hours after UVB irradiation of human skin
12.10	T.B.Fitzpatrick	P.U.V.A.

	Lunch
13.30	Coaches leave Royal College of Physicians for visit to the National Maritime Museum, Greenwich
17.45	Coaches arrive back in London
Golf	The Dowling–McGaw Cup at Mid-Surrey Golf Club, Old Deer Park, Richmond. Please book on the application form. Do not book lunch at the College.
Sailing	The Bowers–Sneddon Sailing Trophy at The Welsh Harp. Please book on the application form. Do not book lunch.
19.30 for 20.00	Annual Dinner at the Savoy Hotel

Saturday 10 July

Clinical Meeting, Normanby College, King's College Hospital, Denmark Hill, SE5 and the Institute of Psychiatry, Denmark Hill, SE5

08.45	Meet at Royal Society of Medicine, 1 Wimpole Street, London for transport to King's College Hospital
13.00	Lunch in Nurses' Dining Room at King's College Hospital

The Social Programme

Thursday 8 July

11.30	Ladies will meet in the entrance hall of the Royal College of Physicians. Coaches will leave punctually for a visit to Kenwood

13.00	Lunch at Kenwood in The Old Kitchen
15.30	Coaches will arrive back at the Royal College of Physicians
18.30–20.00	President's Reception at the Royal College of Obstetricians & Gynaecologists, Sussex Place, Regent's Park

Friday 9 July

13.30	Coaches will leave the Royal College of Physicians for the 1776 Exhibition in honour of the Bicentenary of the Declaration of Independence which is being held at the National Maritime Museum at Greenwich
17.45	Coaches arrive back at the Royal Society of Medicine
19.30 for 20.00	Annual Dinner at The Savoy Hotel—use River Entrance

Saturday 10 July

12 noon	Coaches leave the Royal Society of Medicine for lunch at King's College Hospital

British Journal of Dermatology (1976) **95**, Supplement 14, 9.

SUMMARIES OF PAPERS

Nerve supply as a stimulator of the growth of tissues, including skin

I.W.WHIMSTER

Department of Dermatology, St Thomas's Hospital, London

It is common for benign excesses of cutaneous tissues in man to be associated with excessive innervation of the tissue concerned. Examples to be demonstrated include certain 'benign tumours' supranumerary digits, traumatic neuromas, etc.

These simple observations inevitably raise the question of which is the cause and which the effect. Does a benign excess of tissue somehow attract to it an increased nerve supply, or does increased innervation on occasions itself promote excessive tissue growth?

It will be shown that in lower vertebrates the nerve supply to tissues, including skin, has a very definite role in promoting the growth of the tissues supplied. Examples to be demonstrated will show:

(1) That the regeneration of amputated tails and digits is dependent on an intact nerve supply.

(2) That there is a quantitative relationship between the neural excess and the amount of tissue growth induced by it.

(3) That supernumerary tails can be caused to develop by diverting the transected spinal cord to the floor of a skin wound.

(4) That 'benign tumours' of skin develop at sites where groups of isolated viable nerve cells have been implanted in the floor of skin wounds.

In these animal models it would seem that excess growth of skin (and other) tissues occurs secondarily in response to increased innervation, and that if innervation is reduced regenerative growth is deficient. The implication is that the nerve supply is the cause and the tissue growth the effect.

Enzyme cytochemical and immunocytological characterization of cutaneous lymphomas

O.BRAUN-FALCO AND G.BURG

Dermatology Clinic, University of Munich, Germany

It was the purpose of our study to provide a functional approach to the characterization and classification of cutaneous lymphomas by means of enzyme cytochemical methods (nonspecific esterases, chloracetate esterase, acid and alkaline phosphatase, leucine aminopeptidase, peroxidase) and immunocytological methods (immunoglobulin bearing (B) lymphocytes, spontaneous rosette forming and anti T cell globulin bearing (T) lymphocytes) to differentiate cells harvested from cutaneous lymphomatous infiltrates.

The cellular infiltrates have been evaluated qualitatively and quantitatively in 164 skin biopsies and 61 suspensions from homogenized tissue in 88 patients with the following diseases: benign lymphocytoma and benign inflammatory reactions from insect bites, specific skin infiltrates in chronic lymphatic leukaemia, specific skin infiltrates in monocytic leukaemia, mycosis fungoides, Sézary's syndrome, reticulosis and reticulosarcoma.

Differentiation and classification of these diseases has been achieved based on their typical enzyme-cytochemical and immunocytological patterns.

Mycosis fungoides with leukaemic transformation

CHRISTINE I. HARRINGTON

Rupert Hallam Department of Dermatology, Hallamshire Hospital, Sheffield

An illustrated clinical case of a patient with mycosis fungoides who presented in November 1973, age 57 years, and died of leukaemia in November 1975. Histology of the skin confirmed the clinical diagnosis of 'plaque stage' mycosis fungoides. Detailed investigations, including bone marrow examination, chest tomography, liver and spleen scan and lymphography, were normal.

Topical steroids and ultra-violet light controlled the skin lesions until May 1974, when prednisolone and cyclophosphamide were given, followed by intermittent radiotherapy to painful lesions. Marked deterioration in September 1975 necessitated treatment with anti-mitotics in conjunction with the Department of Oncology. In November 1975 the patient developed a streptococcal septicaemia, a dramatic fall in platelet count and, over a period of 3 days, a rise in white cell count from 11,000/mm^3 to 25,000/mm^3. He died 5 days later with a white count of 40,000/mm^3 of which 57% were blast cells and 7% were myelocytes. The mononuclear cells had a primitive, bizarre morphology.

Post-mortem examination revealed mycosis cell infiltrates in the liver, spleen, thymus, lymph nodes, skin and bone marrow.

A definite diagnosis of mycosis fungoides, with exclusion of systemic involvement at the onset, was achieved. Bone marrow involvement and leukaemic transformation in mycosis fungoides are rare. Was this due to marrow infiltration producing a 'spill-over' of primitive cells into the blood, did a true leukaemic transformation occur and was chemotherapy a factor in producing the leukaemic change?

Trimethylaminuria or fish odour syndrome in a child

ROBIN MARKS, M.W.GREAVES, D.DANKS AND V.PLUMMER

Institute of Dermatology, London and Royal Children's Hospital, Melbourne, Australia

The patient, a boy aged 13 years, attended in April 1975 with a 7-year history of a foul smelling body odour. He described this as a 'fish smell' and it had caused considerable disruption in his social life at school and at play. The smell was noticed in his clothes, sweat, breath and urine. His mother felt that the smell had been worse since reaching puberty.

There was no past history of drug ingestion and no family history of unusual body odour. No other history of systemic disturbance was obtained. Examination of the patient revealed a mildly obese but otherwise normal boy of 13 years. There was an obvious, unpleasant, fishy odour present in his clothes, hair, skin and breath. It was more pronounced in areas of increased sweating, e.g. axillae. There was no discoloration of clothing with sweat. No other abnormality was found on systemic examination.

Bacterial examination of the skin revealed normal flora in the groin, chest, axillae, palms and soles. Urine microscopy and culture revealed no abnormality. Examination of the patient's urine by standard chromatography for amino acids revealed a normal excretion pattern. Intelligence testing showed a score within the normal range for age, with no evidence of brain malfunction.

Gas chromatographic examination of the urine both before and after a high choline diet revealed an excessive amount of trimethylamine. Examination of the urine from family members (mother, father, 2 brothers, 1 sister) under choline load did not reveal the presence of trimethylamine.

The patient was treated initially with a low choline diet. There was an immediate decrease in the overt smell and after 3 weeks on the diet, the patient and his family were unaware of any smell remaining. However, by careful examination it was just possible to detect the presence of the trimethylamine odour on his breath. Gas chromatography of his urine revealed that trimethylamine was still present.

Trimethylamine is the chemical responsible for the characteristic odour of certain fish undergoing bacterial decomposition. In man it is liberated in the gut by bacterial action on dietary choline. It is absorbed and normally completely converted to trimethyl-N-oxide in the liver. If this does not occur, the free trimethylamine base appears in the urine, sweat and breath.

Trimethylaminuria is said to be an extremely rare condition. It has only been described in one patient in the world literature (Humbert *et al.*, 1970). The case described by Humbert *et al.* has been shown to have a decrease in the activity of microsomal mixed-function oxidase responsible for the conversion of trimethylamine in the liver (Higgins *et al.*, 1972).

Choline is the lipotropic factor in the absence of which fatty degeneration of the liver occurs. However, it is possible to lower the intake of choline to a minimal level safely, and in our patient this helped to control the overt clinical manifestation of his illness. Similarly constipation was avoided to prevent excessive bacterial breakdown of the choline which the patient did receive in his diet.

There was no positive past family history and biochemical studies of members of the family are in progress but incomplete. We are therefore unable to determine whether this is an inherited disorder of metabolism. The possibility clearly arises that trimethylaminuria may be more common than the literature suggests, especially in patients with liver disease and chronic intestinal dysfunction, and even in some apparently normal subjects following dietary choline provocation.

REFERENCES

HUMBERT, J.R., HAMMOND, K.B., HATHAWAY, W.E. MARCON, J.G. & O'BRIEN, D. (1970) Trimethylaminuria: fish odour syndrome. *Lancet*, **ii**, 770.

HIGGINS, T., CHAYKIN, S., HAMMOND, K.B. & HUMBERT, J.R. (1972) Trimethyl-N-oxide synthesis: a human variant. *Biochemical Medicine*, **6**, 392.

Measurement of dermal atrophy induced by topical steroids using a radiographic technique

M.P.JAMES, M.M.BLACK AND C.G.SPARKES

St Thomas' Hospital, London, and Glaxo Laboratories Limited, Greenford, Middlesex

Dermal atrophy following the long term application of topical steroids is well recognized. A trial was undertaken to assess the use of a radiographic technique for quantifying dermal atrophy induced by topical steroids (Black, 1969).

Method. Twenty normal adult white males between 25 and 40 years old were divided into four groups. Each person was given a coded syringe containing a control steroid, fluocinolone acetonide 0·025% cream (Synalar), and another coded syringe containing one of the four following test steroids: flurandrenolone 0·0125% cream (Haelan); clobetasone butyrate 0·05% cream (Molivate); hydrocortisone 17-butyrate 0·1% cream (Locoid); hydrocortisone 1% cream. A standard amount (0·5 ml) of the control steroid was rubbed into the middle one third of the radial aspect of one forearm twice daily; the same amount of the test steroid was rubbed into the same site on the other arm. X-rays of the forearm skin were taken before application and at monthly intervals for 3 months. Coded X-rays were measured by two observers.

Results. Significant (>0·1 mm) dermal atrophy was seen with the control preparation in the majority of volunteers. However, variation in the control response was such that comparisons between groups could not be made. With the exception of the hydrocortisone group, where no atrophy could be detected, significant responses were seen in some individuals with all the test preparations. There were more instances of significant atrophy at 3 months than at 1 month.

Comment. Dermal atrophy has been shown to occur with fluocinolone 0·025%, hydrocortisone 17-butyrate 0·1%, clobetasone butyrate 0·05% and flurandrenolone 0·0125% using this radiographic technique. Hydrocortisone 17-butyrate has previously been thought not to suffer from the same atrophogenic properties as fluorinated steroids (Sneddon, 1973). This preliminary study shows that dermal atrophy does occur with this steroid. Taking X-rays at 1 month only would have underestimated the number of volunteers who subsequently developed atrophy.

This technique is a reliable, quantitative method for measuring dermal atrophy. It would be possible to adapt this technique to study other sites, e.g. the face; and to compare atrophogenic properties of different steroids.

REFERENCES

BLACK, M.M. (1969) A modified radiographic method for measuring skin thickness. *British Journal of Dermatology*, **81**, 661.

SNEDDON, I. (1973) A trial of hydrocortisone butyrate in the treatment of rosacea and perioral dermatitis. *British Journal of Dermatology*, **89**, 505.

Pseudopyogenic granuloma: the histopathogenesis in the light of ultrastructural studies

R.A.J.EADY, T.COWEN AND E.WILSON JONES

Institute of Dermatology, Homerton Grove, London

The clinical and histological features of pseudopyogenic granuloma (PPG) are well recognized (Wilson Jones & Bleehen, 1969) but the pathogenesis is unknown. Furthermore, little is known about the nature and origin of the abnormal hypertrophic endothelial cells that characterize PPG.

Typical PPG vessels in three lesions were studied by electron microscopy. The ultrastructural findings were correlated with histological material from thirty-six patients and with enzyme histochemical studies (ten lesions).

Light microscopy revealed peculiar endothelial cells with voluminous cytoplasm which protruded into the vascular lumina and demonstrated marked respiratory and hydrolytic enzyme content. Such features are not known to occur in other vascular proliferative disorders. Electron microscopy revealed two distinct types of endothelial cell. Compared with normal endothelium, the predominant cell type contained a great abundance of cytoplasmic organelles including mitochondria, granular endoplasmic reticulum, Golgi complex, free ribosomes and lysosome-like bodies. The less common cells were smaller and contained tightly packed microfilaments together with numerous intracytoplasmic and pinocytotic vesicles. Both types of endothelial cell possessed sparse Weibel Palade bodies. Intercellular junctions were also infrequent.

These findings indicate that the endothelium in PPG vessels is highly active metabolically. Ultrastructurally, the predominant type of endothelial cell shows features of regeneration or proliferation, but the stimulus inducing this pathological change remains to be elucidated.

REFERENCE

WILSON JONES, E. & BLEEHEN, S.S. (1969) Inflammatory angiomatous nodules with abnormal blood vessels occurring about the ears and scalp (pseudo or atypical pyogenic granuloma). *British Journal of Dermatology*, 81, 804.

Malignant melanoma. A comparison of cellular fine structure in invasive nodules of different histogenetic type

J.A.A.HUNTER,* S.ZAYNOUN,* W.D.PATTERSON,* R.M.MACKIE,† A.J.COCHRAN† AND S.S.BLEEHEN‡

* *University Department of Dermatology, The Royal Infirmary, Edinburgh,* † *University Departments of Dermatology and Pathology, The Western Infirmary, Glasgow, and* ‡ *Rupert Hallam Department of Dermatology, Sheffield*

It is now appreciated that there are three major types of invasive malignant melanoma—lentigo maligna melanoma (LMM), superficial spreading melanoma (SSM) and nodular melanoma (NM). All differ in their mode of onset, course and prognosis, and each has a distinctive clinical and histological appearance (McGovern *et al.*, 1972.)

Recent publications have also detailed certain ultrastructural features thought to be characteristic of the histogenetic type (Clark *et al.*, 1972; Mishima & Matsunaka, 1975). However the number of specimens examined was small, and the conclusions may have been biased by observer knowledge of the tumour type.

Forty surgically excised specimens of malignant melanoma were studied. Case summaries, clinical photographs and light histological material were sent to Glasgow (R.M. and A.C.) and the histogenetic type of each tumour determined.

Electron-microscopy of tissue obtained from the invasive nodule was carried out in Edinburgh (J.H. and S.Z.) so that neither worker was aware of the histogenetic type of tumour being examined. At least two blocks from each tumour were studied, and a minimum of thirty random photographs taken. The electron micrographs were then analysed independently in Edinburgh (J.H.) and Sheffield (S.B.), both workers still being unaware of the tumour type. Particular attention was paid to melanosomal morphology.

The code was then broken and the ultrastructural features correlated with the histogenetic type.

Results. Types of tumour in series: LMM, 14; SSM, 14; NM, 9; metastatic skin deposit, 3.

Most tumour cells contained melanosomes which were either ellipsoidal or spheroidal. The morphology of the ellipsoidal profiles was usually similar to that of normal melanosomes. Spheroidal melanosomes were of the abortive, granular or lamellar type (Clark *et al.*, 1972). Tumour cells of LMM contained numerous ellipsoidal melanosomes whilst those of SMM, NM and metastatic deposits contained only occasional ones. All varieties of spheroidal melanosomes were seen in each histogenetic type, though granular profiles were less common in LMM.

Thus the ultrastructural appearance of LMM is reasonably characteristic, but there are no obvious differences in melanosomal morphology in the nodules of SMM, NM and metastatic deposits. These results can be explained most easily on the basis of subcellular dedifferentiation due to the malignant process.

REFERENCES

CLARK, W.H. JR., HEGGELER, B.T. & BRETTON, R. (1972) *Electron Microscope Observations of Human Cutaneous Melanomas Correlated with their Biologic Behaviour in Melanoma and Skin Cancer* (Ed. by W.H.McCarthy), p. 122. Government Printer, Sydney.

MCGOVERN, V.J., MIHM, M.C. JR., BAILLY, C., BOOTH, J.C., CLARK, W.H. JR., COCHRAN, A.J., HARDY, E.G., HICKS, J.D., LEVENE, A., LEWIS, M.G., LITTLE, J.H. & MILTON, G.W. (1972) The classification of malignant melanoma and its histologic reporting. *Cancer*, **32**, 1446.

MISHIMA, Y. & MATSUNAKA, M. (1975) Pagetoid premalignant melanosis and melanoma: differentiation from Hutchinson's melanotic freckle. *Journal of Investigative Dermatology*, **65**, 434.

Lentigo-maligna melanoma: an ultrastructural study

S.S.BLEEHEN, LISA HARTLEY AND JENNIFER SENIOR

Department of Dermatology, Hallamshire Hospital and University of Sheffield

Electron microscopic studies have been carried out on multiple biopsies from eight lentigo-maligna melanomas, to determine the fine structure of the 'surround' and 'nodule' cells of this type of melanoma.

The 'surround' cells of the centrifugal growth phase were almost invariably Type I, using the classification of Clark, Heggeler & Bretton (1972). These cells contained mainly ellipsoidal melanosomes, whose internal structure was essentially normal. Auto-phagocytosis of melanosomes was common in these cells with membrane bounded aggregates of melanosomes. In addition to these melanosomal complexes there were lysosomal structures containing mitochondrial debris as well as lamellated bodies. The 'nodule' cells were mainly Type I, but melanosomal and nuclear abnormalities were more frequent than in the 'surround' cells. There were also a few more undifferentiated Type II and Type III cells to be found. These cells contained spherical melanosomes which were either abortive melanosomes containing one or two coiled melanofilaments with no cross striations or membrane bounded organelles with a granular internal matrix of varying electron density.

The ultrastructural findings in lentigo-maligna melanoma sharply contrast with and differ from those of the superficial spreading (Pagetoid) and nodular types of melanoma.

REFERENCE

Clark, W.H. Jr., Heggeler, B. & Bretton, R. (1972) Electron microscopic observations of human cutaneous melanomas correlated with their biologic behaviour. In: *Melanoma and Skin Cancer* (Ed. by W.H.McCarthy) p. 121. V.C.N.Blight, Sydney.

Cutaneous tuberculosis of the nose with unusual clinical and histological features leading to delay in diagnosis

A.P.Warin and E.Wilson Jones

St John's Hospital for Diseases of the Skin, London WC2

The details of five patients will be presented, all of whom had cutaneous tuberculosis of the nose. Unusual clinical features and nonspecific histology led to a delay in the diagnosis of four of them.

Case I: Mrs J.U., aged 52 years. History. When she was first seen at St John's Hospital she had had a 25-year history of atrophic rhinitis. She had had several operations to correct a deviated septum and this was followed by collapse of the septum.

Ten years before presenting she started to get crusting around the nostrils. She was seen by a dermatologist who considered a diagnosis of lupus vulgaris, but histology was entirely non-specific. Some months before she presented she started to develop extension of the erythema and crusting all over the nose. The nose was red and indurated and was studded with pustules. A diagnosis of rosacea was considered and histology was consistent with this, but there was no response to 3 months of oxytetracycline. She had had pulmonary tuberculosis in 1943 and her chest X-ray showed old healed T.B. She gave a history of recurrent nodules on the calves for 20 years. These were sometimes painful and occasionally ulcerated.

Investigations. Biopsy nose 1962, non-specific inflammation; Biopsy nose 1972, compatible with rosacea; Biopsy leg 1972, nodular vasculitis; Mantoux test 1 in 10,000 positive; Culture (1972) of biopsy material from the nose revealed a scanty growth of acid alcohol fast bacilli.

Treatment. Para-aminosalicylic acid and isoniazid produced an excellent response of both her nose and her nodular vasculitic lesions.

Case II: Mrs E.B. aged 66 years. History. She was first seen at St John's Hospital with a 2-year history of wasting and erythema around the nose and the tip of the nose. She had been seen by a dermatologist who reported that the lesion had no granulomatous features then, but a biopsy was not done.

She presented with a clinically granulomatous lesion of the nose with some possible scarring. There was a nasal perforation. There was no previous history of T.B. and she was not on systemic corticosteroids.

Investigations. Histology showed an ulcerated lesion with epithelioid follicles and giant cells.

Mantoux test was positive at 1 in 10,000; Chest X-ray, no abnormality; Culture of skin biopsy for A.F.B.'s was negative; Guinea-pig inoculation was negative.

Treatment. Response to para-aminosalicylic acid and isoniazid was excellent.

Case III: Mrs W.P. aged 74 years. History. She had a 6-month history of the development of crusted lesions around the nose and upper lip and left cheek, which gradually became a very proliferative, vegetating granuloma, associated with a large anterior nasal perforation. She was not on systemic corticosteroids. She had no previous history of tuberculosis.

Investigations. Histology showed warty hyperplasia with underlying tuberculoid granuloma. There was no caseation and no A.F.B.'s were seen.

Mantoux test: markedly positive at 1 in 10,000; Chest X-ray, no abnormality; Bacteriology, *Staphylococcus aureus* and acid fast bacilli isolated on culture of biopsy material.

Treatment. The response to rifampicin, ethambutol and isoniazid was excellent.

Case IV: Mrs E.B. aged 71 years. History. She presented with a 6-month history of ulceration and inflammation of the tip of the nose. She had had a tuberculous gland in the neck at the age of 20 years.

Investigations. Three separate biopsies were entirely non-specific. Culture and guinea-pig inoculation were negative for A.F.B.'s. There was a very heavy growth of *staphylococcus aureus.*

Treatment. Because of the development of apple jelly nodulation, she was treated with para-amino salicyclic acid and isoniazid. The response was excellent.

A fifth case will be discussed.

Comment. These cases are discussed to emphasize how difficult it can be to diagnose tuberculosis of the skin when it involves the nose. Delay in diagnosis can lead to unpleasant scarring. There are cases when a trial of anti-tuberculous therapy seems to be entirely justified in spite of not having a definite tuberculous clinical or histological picture, even if the culture is negative too.

The production of antibiotics by *Trichophyton* and *Epidermophyton* species

W.C.NOBLE, YVONNE M.CLAYTON, NAGWAN YOUSSEF, G.HOLT AND C.WYBORN

Institute of Dermatology, and Polytechnic of Central London

Dermatophytes have long been known to produce metabolites which restrict the growth of bacteria. In the past, attention has been concentrated on 'penicillin' production *in vitro* and it is well established that *Trichophyton mentagrophytes* and *Epidermophyton floccosum* produce benzyl penicillin, and 6-aminopenicillanic acid. Other antibiotics have been hinted at—the most developed of which was the steroid type antibiotic reported by Wallerstrom.

Production of antibiotics *in vivo* has not yet been described in human studies but such production would be important for two reasons. One is that it would provide a source of antibiotic resistant organisms without the necessity of postulating the soil as a source of antibiotics. The second is that it would complete the chain of evidence suggesting that allergy to penicillin in the apparent absence of therapeutic penicillin is related to lesions of tinea.

Joint studies between workers at the Institute of Dermatology and the Polytechnic of Central London have shown that clinical isolates of dermatophyte fungi produce antibiotics when grown in a fermentation medium designed for maximal production of benzyl penicillin. All eight strains of *T. mentagrophytes* tested produced antibiotic, six of eleven strains of *T. rubrum* and four of eight strains of *E. floccosum* also produced antibiotic. In this medium several antibiotics were produced. These include benzyl penicillin, 6-aminopenicillanic acid, streptomycin and an antibiotic resembling azalomycin.

Using the technique devised by A.G.Knight for growing dermatophyte fungi on human stratum corneum *in vitro*, both Trichophyton species have been proved to produce antibiotic under conditions resembling those found in the host. So far *Epidermophyton* strains have not produced antibiotic on stratum corneum.

Taxonomy of aerobic cutaneous diphtheroids

D.G.PITCHER

Institute of Dermatology, London

The aerobic diphtheroids of human skin have historically been considered as members of the genus *Corynebacterium*. It is now generally considered that a characteristic of the mammalian corynebacteria is the possession of a cell wall composition based on *meso*-diaminopimelic acid (*meso*-DAP) and arabinogalactan.

In screening over 500 aerobic diphtheroids for these compounds it was found that approximately 60% of isolates possessed the expected cell wall type and counts of the flora from nine skin sites on eighteen subjects revealed that these corynebacteria were the commonest diphtheroids isolated from most sites, the intertriginous areas being particularly rich in them.

Coryneform organisms morphologically resembling the diphtheroids but without DAP in the cell wall constituted 12% of the isolates; counts were generally low, there was a variety of cell-wall patterns included in this group and the commonest sites of isolation were on the more exposed areas such as the forehead. It is suggested that the former group represent resident members of the cutaneous flora and the latter, transient or contaminating coryneforms from the environment.

A third group of diphtheroids (14%) possessed *meso*-DAP with galactose as the only detectable sugar; these strains were isolated in low but consistent numbers and showed no site preference. It is considered that this group represent a small but resident proportion of the flora.

The relationship of the various cell wall types was investigated by numerical taxonomy. Seventy-one isolates spanning the spectrum of cell wall compositions and biotypes were compared to sixty-three marker strains using 100 test parameters.

From the resulting dendrograms, two major and two minor clusters were seen at the 76% similarity level including all the *meso*-DAP/arabinogalactan types (forty-nine strains).

Strains of the *meso*-DAP/galactose type also fell within the four groups and formed three distinct subgroups. Those strains not possessing DAP were excluded from the four clusters and were loosely ranged among marker strains of non-human origin.

The conclusion drawn from these results is that not all aerobic diphtheroids from human skin should be regarded as members of *Corynebacterium*. Though the major proportion of isolates may be, a small but consistent proportion of the diphtheroid population are probably distinct from the *Corynebacterium sensu stricto*, and possess *meso*-DAP and galactose in their cell walls.

The absence of DAP walls in coryneforms derived from the more exposed areas of skin indicates that they are probably transient bacteria from the environment.

The significance of various wall types in relation to minor human disease such as erythrasma and pitted keratolysis is being sought.

A non-acne naevus. A clinical and laboratory study

W.J.CUNLIFFE, *M.B.HODGKINS AND R.EAD

*Department of Dermatology, General Infirmary, Leeds and * Department of Dermatology and Biochemistry, University of Glasgow*

We report a patient with an unusual naevus, investigations of which give support to some of the ideas behind the understanding of acne. The patient was a 17-year-old boy with extensive acne on the back, except for two sets of symmetrical sparing, one set below the scapulae and the other on the shoulders. The lack of acne was not due to fewer glands in the naevus but the sebaceous glands in the naevus were smaller and produced 33% less sebum.

In vitro incubation of sebaceous glands from the naevus and acne skin with ^{3}H-testosterone suggested that this decreased sebum excretion is probably due to a decreased rate of conversion of testosterone to 5α-dihydrotestosterone in the naevus as compared to acne skin.

Micro-biological examination of the skin surface showed no difference but there were fewer *Corynebacteria acnes* in the ducts of the naevus. Surface microscopy of the skin and microscopic examination of skin surface biopsy showed more keratin in the acne area. There is some evidence to suggest that squalene and wax ester are comedogenic and we found an increased squalene and wax ester content in lipids from the acne skin. The percentage of the free fatty acids was the same in both the acne and naevus areas and this may add weight to the growing body of evidence to suggest that fatty acids are not so important in acne as was believed several years ago.

Pathology of lichen striatus

W.M.STEWART

Hôpital Charles-Nicolle, Rouen, France

The striking feature of lichen striatus (LS) is the cellular infiltration of the dermal papillae roughly similar to lichen planus (LP) from which it differs in three points:

(1) The infiltrate is usually less dense occupying three or four adjacent papillae. (2) Histiocytes are mixed with lymphocytes and associated with oedema of the entire papilla. (3) At the top of the papillae the infiltrate makes contact with the epidermis extending more or less into the lower layers, without the cleft frequently seen in LP. At the base of the papillae the limit is not horizontal but goes down lower in the mid-dermis, sometimes in continuity with the perivascular infiltration of the upper dermis.

In very young lesions, the infiltration might be very dense, obscuring the limits of the upper dermis and epidermis. In less inflammatory (older ?) lesions the vanishing infiltration unmasks a feature that seems particular to the disease: *small and clear cavities*, breaking from place to place the continuity of the basal layer, empty or partially occupied by cellular ghosts (Civatte bodies) and melanin granules. This tendency to a *transient destruction of the germinative layer* (basal cells and melanocytes) is a useful sign of LS that explains clinical symptoms such as LS albus, transient leucoatrophy of the nail. It coexists frequently with the other features described above even on the same slide.

Further studies in herpes gestationis

J.A.Carruthers and M.M.Black

St John's Hospital for Diseases of the Skin, London

Previous reports (Provost & Thomas, 1973; Carruthers & Black, 1975) have shown herpes gestationis (HG) to be a disease of pregnancy which is similar to, but different from, bullous pemphigoid. A circulating factor is present in these patients which is capable of depositing complement on the basement membrane zone (BMZ) of normal human skin. This paper reports further studies to characterize this factor as well as hormonal studies on one of our patients.

We have studied the serum of two post-partum patients with severe, persistent HG. Using indirect complement immunofluorescence (ICIF) as an assay, the following properties of the HG factor have been demonstrated:

(1) It deposits C3 complement at the BMZ of normal human skin (but not guinea-pig oesophagus), whereas immunoglobulin deposits at the BMZ cannot be demonstrated by routine indirect immunofluorescence. (2) Activity is heat stable (56°C for ½ h) provided fresh normal human serum is added as a source of complement. (3) Complement fixation is dependent on the 'classical' pathway because no C3 is fixed from C2-deficient human serum or C4 deficient guinea-pig serum when these are used as the complement source. (4) On DEAE cellulose and on G200 Sephadex the complement fixing activity elutes with the IgG fraction. (5) Absorption of IgG from the DEAE cellulose eluate using a batch technique removed the C3 fixing activity.

Hormonal assays were performed on one post-partum patient throughout a menstrual cycle. These were correlated with the titre of anti-BMZ activity by ICIF. Abnormalities of the hormonal cycle were present but there was no significant change in the titre of ICIF activity. At this time the patient was experiencing premenstrual exacerbation of pruritus and the HG rash.

These results indicate that the HG factor is an IgG immunoglobulin particularly effective in fixing complement via the classical pathway. It is also suggested that the clinical expression of the disease is dependent not only on the presence of the HG factor but also on a hormonal imbalance.

REFERENCES

CARRUTHERS, J.A. & BLACK, M.M. (1975) Immunopathological findings in herpes gestationis. *British Journal of Dermatology*, **93**, Suppl. 11, 17.

PROVOST, T.T. & TOMASI, T.B. (1973) Evidence for complement activation via the alternative pathway in skin diseases. I. *Journal of Clinical Investigation*, **52**, 1779.

Digital ischaemia due to vascular anomalies

NEVILLE R.ROWELL

General Infirmary at Leeds

Three patients, aged 22, 47 and 60 years, with painful cyanosis of several fingers have been studied. Investigations for primary systemic disease were negative. All the patients smoked 20 or more cigarettes a day but none had been on any drugs. The peripheral pulses were present.

Arteriography revealed vascular anomalies of the palmar arch. In the patient with ischaemia of the thumb and index fingers the palmar arch was almost exclusively supplied by the ulnar artery; in the patient with ischaemia of the ring and fifth finger, the arch was supplied by the radial artery; in the third patient with ischaemia of all fingers except the thumb, there was poor blood supply from both the radial and ulnar arteries. Bilateral arteriography in one patient showed the changes to be similar in both hands. Evidence from other patients suggests that similar vascular anomalies occur in the feet.

It is proposed that arteriography is indicated in all patients presenting with persistent ischaemia of the fingers and toes when the peripheral pulses are present and systemic disease has been excluded.

Photochemotherapy. A study of its efficacy in fifty patients suffering from psoriasis and other dermatoses

T.LAKSMIPATHI, P.GOULD, B.E.JOHNSON, L.A.MACKENZIE AND W.FRAIN-BELL

University of Dundee

Photochemotherapy, in the form of the oral administration of 8-methoxypsoralen followed by exposure of the skin to long ultraviolet irradiation (UVR), has been shown to be effective in the treatment of certain dermatoses and in particular psoriasis (Parrish *et al.*, 1974).

In a group of fifty subjects suffering from psoriasis, parapsoriasis, and mycosis fungoides, the effect of photochemotherapy has been assessed over a period of 1 year. Except for two patients with psoriasis, a significant clinical improvement was demonstrated. A regime of maintenance therapy is being defined.

The equipment used consisted of conventional long UVR tubes mounted in specially constructed cubicles.

REFERENCE

PARRISH, J.A., FITZPATRICK, T.B., TANENBAUM, L. & PATHAK, A. (1974) Photochemotherapy of psoriasis with oral methoxsalen and longwave ultraviolet light. *New England Journal of Medicine*, **291**, 1207.

Effects and side-effects of 8-methoxypsoralen-blacklight-therapy in more than 1500 patients

GERHARD WEBER

Skin Clinic of City Hospital Nuernberg/FRG

Since 1972 about 1500 in- and outpatients have been treated with the combined 8-methoxypsoralen and blacklight therapy (for details of the technique see Weber, 1974) and also with new blacklight lamps. This therapy has proved to be *very good* in psoriasis, acne, bacterial disseminated eczema, parapsoriasis en plaques, mycosis fungoides I–III, herpes zoster, and *good* in herpes simplex and papular urticaria.

We have *not* had *sufficient experience* with vitiligo, pityriasis lichenoides, disseminated lichen planus, carcinomas.

This therapy did not show *any effect* with atopic dermatitis or with mycotic eczema.

The rate of relapse in psoriasis, the side effects and their exogenous as well as endogenous causes will be discussed; parallel examinations of metabolism will be demonstrated. The organization of our radiation section for 120 to 150 patients treated every day and the geographical extension of the blacklight therapy in our country will be explained.

REFERENCE

WEBER, G. (1974) Combined 8-methoxypsoralen and black light therapy of psoriasis. Technique and results. *British Journal of Dermatology*, **90**, 317.

Increase in prostaglandin E_2 and $F_{2\alpha}$ in inflammatory exudate at 4–48 h after UVB irradiation of human skin

A.KOBZA BLACK, M.W.GREAVES, C.N.HENSBY, A.PAZZAGLIA AND N.PLUMMER

Institute of Dermatology, London

Little is known of the relationship of prostaglandin (PG) activity and other mediators to the time course of ultraviolet (UV) induced inflammation. A suction blister method was used to obtain inflammatory exudate from human skin 4–48 h after irradiation with UVB (3 × MED). Results in normal (control) and irradiated skin of the same subjects were compared. Preliminary pharmacological analysis for PG, histamine and bradykinin activity was carried out by cascade superfusion bioassay. PGs were further characterized by solvent extraction and gel partition chromatography. $PGF_{2\alpha}$ was also measured by radio-immunoassay. At 24 h after irradiation there was a 20-fold increase in total PG activity (bioassayed in terms of PGE_2) in UVB irradiated skin compared with control. Gel partition chromatography showed an approximately 20-fold increase in PGE_2 and $PGF_{2\alpha}$ activity compared with control. Further confirmation of these findings was obtained by radio-immunoassay which revealed a 3-fold increase in $PGF_{2\alpha}$. Increase in PG activity was detectable 4 h after irradiation but there was little or no increase in activity at 48 h compared with control. No increase in histamine or bradykinin activity was detected at any time. The correlation of PGE and PGF activity with the time course of the inflammatory response to UVB lends support to the view that prostaglandins play an important role in the mediation and regulation of the inflammatory response to UV.

British Journal of Dermatology (1976) **95**, Supplement **14**, 23.

CASE REPORTS

(1) Juvenile bullous pemphigoid

DR M.SKEETE AND PROFESSOR M.W.GREAVES

St John's Hospital for Diseases of the Skin, London WC2

M.F., female, aged 12 years. School-girl.

History. In June 1975, she presented with a 10-month history of a pruritic bullous eruption, which had not responded to a trial of sulphapyridine 0·5 g three times daily for 2 weeks followed by dapsone 100 mg/day for 2 weeks.

Examination. There was a generalized vesiculo-bullous eruption arising on a background of figurate erythema, predominantly situated on the face, forearms and legs and there were erosions on her lower lip. There were no other abnormal physical signs.

Investigations. Routine investigations were normal apart from levels of serum complement components which showed an elevated C3 of 263 mg% (normal range 75–165 mg%) and C7 of 170% of standard reference serum (normal range 80–140% standard reference serum).

Biopsy of a maculopapular lesion showed no blister but nuclear debris at the papillary tips suggestive of dermatitis herpetiformis.

Immunofluorescence. Direct immunofluorescence showed a linear basement membrane zone fluorescence to IgG, C3 and a weak fluorescence to IgM (Fig. 1). Indirect immunofluorescence showed basement membrane zone staining to an initial titre of 1/320. Further titres closely correlated with the clinical condition (Fig. 2).

Treatment. Her pemphigoid was controlled with prednisone 500 mg/day which was reduced to 50 mg/day, when she was discharged clinically clear 12 weeks after admission. The current dose is prednisone 20 mg/day.

Comment. Bullous pemphigoid which is usually a disease of the elderly can occur in children as seen in eight cases reported between 1970 and 1973 (Bean *et al.*, 1970; Hashimoto, 1970; Fincher *et al.*, 1971; Jablonska *et al.*, 1971; Esterly *et al.*, 1973; Faber *et al.*, 1973). Clinically, the disease in childhood resembles that of the adult with two exceptions:

(1) An increased incidence of oral lesions (Bean & Jordon, 1974).

(2) A higher dose of corticosteroids is usually needed for control (Rook *et al.*, 1972).

Immunofluorescent studies can be used to establish the diagnosis and differentiate bullous pemphigoid from the other bullous dermatoses of childhood. *In vivo* bound basement membrane zone antibody was shown in all eight of the above reported juvenile cases. This is similar to immunofluorescent findings in adults, where basement membrane zone deposits of IgG and C3 are diagnostic of bullous pemphigoid and found in virtually all cases (Beutner *et al.*, 1970; Jordon *et al.*, 1971). Circulating basement membrane zone antibodies, which are specific for pemphigoid, (Beutner *et al.*, 1973) are also found in children as was seen in five of the eight reported cases and one of the children reported by Chorzelski *et al.* (1975). Unlike this present case, titres usually show only a rough correlation, if any, with disease activity (Sams & Jordon, 1971; Beutner *et al.*, 1973).

The complement levels in serum are normal or slightly raised (Katz *et al.*, 1970; Beutner *et al.*,

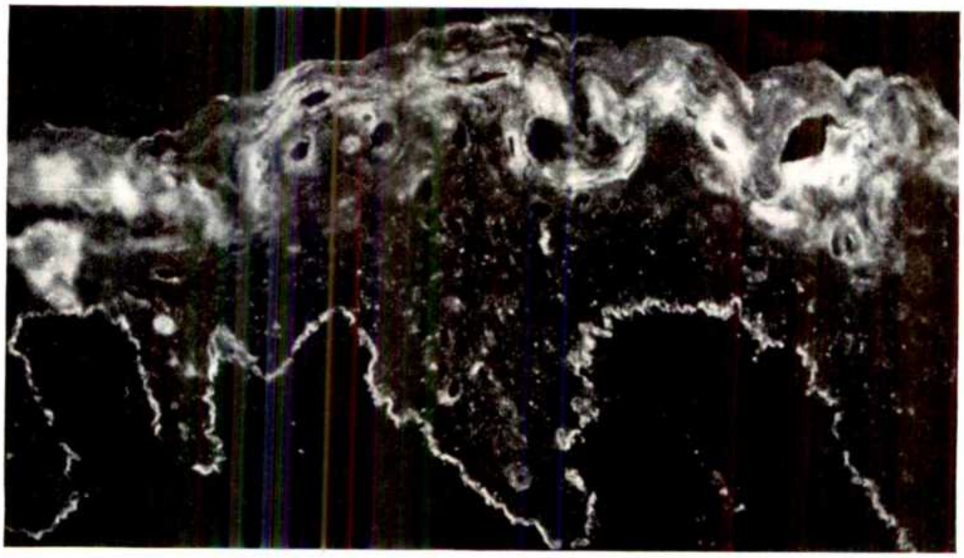


FIGURE 1. Direct immunofluorescence to IgG showing basement membrane zone fluorescence.

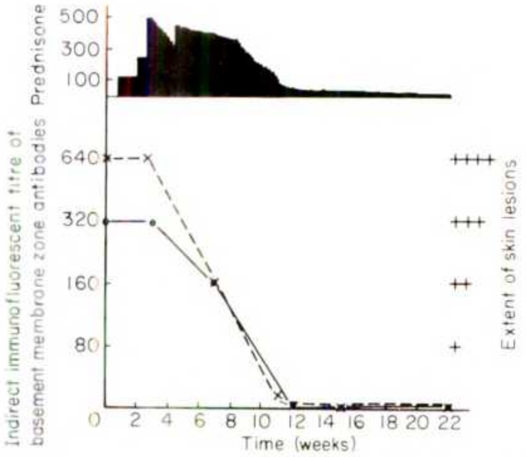


FIGURE 2. Indirect immunofluorescent titres in relation to the clinical condition and its response to therapy. —— Antibody titre; - - - - extent of skin lesions.

1973; Jordon *et al.*, 1973; Jordon, 1975), although they are lower in the blister fluid (Jordon *et al.*, 1973). The serum concentration appears insensitive to the peripheral process (Katz *et al.*, 1970) suggesting a local activation of complement in the lesion (Beutner *et al.*, 1973).

REFERENCES

BEAN, S.F., GOOD, R.A. & WINDHORST, D.B. (1970) Bullous pemphigoid in an 11-year-old boy. *Archives of Dermatology*, **102**, 205.

BEAN, S.F. & JORDON, R.E. (1974) Chronic non hereditary blistering disease in children. *Archives of Dermatology*, **110**, 941.

BEUTNER, E.H., CHORZELSKI, T.P., BEAN, S.F. & JORDON, R.E. (1973) *Immunopathology of the skin: labelled antibody studies*, pp. 220 and 363–365. Dowden, Hutchinson & Ross Stroudsburg, Pennsylvania.

BEUTNER, E.H., CHORZELSKI, T.P. & JORDON, R.E. (1970) *Autosensitization in pemphigus and bullous pemphigoid*, pp. 96–98. Charles C. Thomas, Springfield, Illinois.

CHORZELSKI, T.P., JABLONSKA, S., BEUTNER, E., MACIEJOWSKA, E. & JARZABEK-CHORZELSKA, M. (1975) Juvenile dermatitis herpetiformis versus 'benign chronic bullous dermatosis of childhood'. Are these immunologic diseases? *Journal of Investigative Dermatology*, **65**, 447.

ESTERLY, N.B., GOTOFF, S.P., LOLEKHA, S., MOORE, E.S., SMITH, R.D., MEDENICA, M. & FUREY, N.L. (1973) Bullous pemphigoid and membraneous glomerulonephropathy in a child. *Journal of Pediatrics*, **83**, 466.

FABER, W.R. & VAN JOOST, T.H. (1973) Juvenile pemphigoid. *British Journal of Dermatology*, **89**, 519.

FINCHER, D.F., DUPREE, E. & BEAN, S.F. (1971) Bullous pemphigoid in childhood. Immunofluorescent studies. *Archives of Dermatology*, **103**, 88.

HASHIMOTO, K. (1970) Paramyxovirus-like inclusions in several skin diseases of unknown etiology, abstracted. *Clinical Research*, **18**, 349.

JABLONSKA, S., CHORZELSKI, T., BEUTNER, E.H. & BLASZCZYK, M. (1971) Juvenile dermatitis herpetiformis in the light of immunofluorescence studies. *British Journal of Dermatology*, **85**, 307.

JORDON, R.E. (1975) Complement activation in bullous skin diseases. *Journal of Investigative Dermatology*, **65**, 162.

JORDON, R.E., DAY, N.K., SAMS, W.M. JR & GOOD, R.A. (1973) The complement system in bullous pemphigoid. Complement and component levels in sera and blister fluid. *Journal of Clinical Investigation*, **52**, 1207.

JORDON, R.E., TRIFTSHAUSER, C.T. & SCHROETER, A.L. (1971) Direct immunofluorescent studies of pemphigus and bullous pemphigoid. *Archives of Dermatology*, **103**, 486.

KATZ, S.I., INDERBITZIN, T.M. & HALPRIN, K.M. (1970) Serum complement (C_3) levels in patients with autoimmune bullous skin diseases. *Archives of Dermatology*, **102**, 368.

ROOK, A., WILKINSON, D.S. & EBLING, F.J.G. (1972) *Textbook of Dermatology*, p. 1318. Blackwell Scientific Publications, Oxford.

SAMS, W.M. JR. & JORDON, R.E. (1971) Correlation of pemphigoid and pemphigus antibody titres with activity of disease. *British Journal of Dermatology*, **84**, 7.

(2) Transient acantholytic dermatosis

DR C.B.BENTLEY-PHILLIPS AND DR G.C.WELLS

St John's Hospital for Diseases of the Skin, London WC2

S.G., male, aged 65 years.

History. In December 1974 he first developed a pruritic rash, predominantly on the upper trunk with the axillae, neck, periumbilical area, upper arms and thighs also involved. At first he had many small blisters, but these no longer occur, although the pruritic rash has persisted, in spite of the use of antihistamines, topical steroids and dapsone.

On examination. Small pin-head vesicles were present originally on the upper trunk, but more recently only excoriated papular lesions have been observed.

Investigations. Blood count, ESR, blood urea, urine and chest X-ray have all been normal, as have the liver function tests (with the exception of a serum bilirubin level of 1·6 mg/100 ml).

Histology. Biopsies showed small suprabasal clefts and acantholysis in multiple discrete foci.

Immunofluorescence. Direct and indirect tests showed no fluorescence.

Comment. This patient appears to correspond to those cases described by Grover under the title 'Transient Acantholytic Dermatosis'. These patients suffered from transient pruritic vesicular or papular lesions, which on histology demonstrated acantholysis and dyskeratosis. Ackerman later broadened the concept to include less transient cases with suprabasilar clefting.

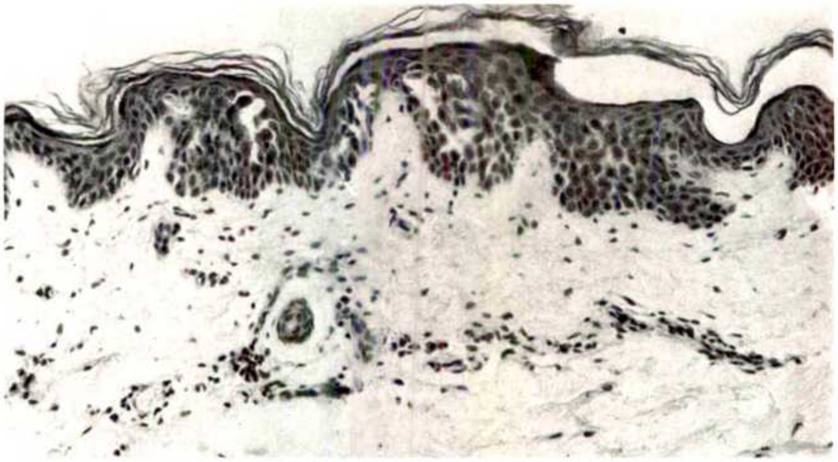


FIGURE 1. Suprabasal cleft and acantholysis.

REFERENCES

GROVER, R.W. (1970) Transient acantholytic dermatosis. *Archives of Dermatology*, **101**, 426.
ACKERMAN, A.B. (1972) Focal acantholytic dyskeratosis. *Archives of Dermatology*, **106**, 702.

(3) Localized pemphigoid

DR G.P.SPARROW AND DR E.J.MOYNAHAN

Guy's Hospital, London

J.I., female, aged 65 years.

History. At the age of 63 years she injured her right lower leg in a fall, causing a wound which took 2 months to heal. One month after healing was complete she developed a large bulla on the leg below the scar. Since then she has had recurrent blisters on this site, and also on an area behind the ankle. Bullae arise either singly or in groups of 2–3, coming up over a few hours, and are preceded by itching and severe pain. They discharge a clear or purulent fluid, become crusted, and heal spontaneously in about a week, without scarring. The interval between attacks varies from 2 to 6 weeks. There have been no blisters elsewhere on the skin or on the mucous membranes. She used to take Sanatogen tablets and phenolphthalein-containing laxatives, but the bullae continued to recur in the same way after ceasing these drugs, and did not appear to be related to them in time.

Examination. There is a triangular atrophic scar on the right lower leg. Inferior to it, and also posterior to the lateral malleolar area, there are areas of purplish erythema and hyperpigmentation, on which arise unilocular bullae 1–3 cm in diameter (Fig. 1).

Investigations. Histological examination: 'a subepidermal bulla is present, containing fibrin, neutrophils and eosinophils. The upper dermis is oedematous and there is a perivascular lymphocytic infiltrate. Consistent with bullous pemphigoid.'

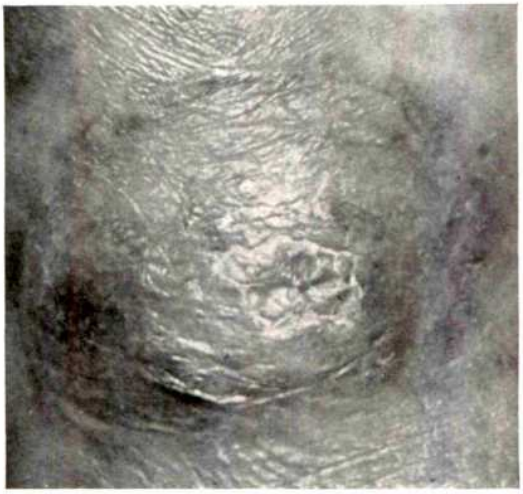


FIGURE 1. Showing a subsiding bulla.

Direct immunofluorescence: positive basement membrane zone fluorescence to IgG and fibrin.

Indirect Immunofluorescence: Anti-basement membrane antibody titre 1:320. Haemoglobin 15 g. White cell count 5800/mm^3, normal differential. ESR 13 mm/h. Antinuclear factor negative. Urea, electrolytes, liver function tests, serum proteins, urinalysis and chest X-rays were normal. HLA antigens 1, W32, 8, W16.

Treatment and progress. Oral prednisolone both in short courses beginning at 40 mg/day and continuously in a dose of 5–15 mg daily did not prevent the formation of new bullae. Intralesional and topical steroids were of some benefit. The condition appears to be improving spontaneously.

Discussion. This patient has sub-epidermal bullae histologically consistent with bullous pemphigoid, and this diagnosis is supported by the findings on direct and indirect immunofluorescence, which seem to exclude the possibility of a fixed drug eruption. Benign mucous membrane pemphigoid may produce bullae on the skin, but they heal with scarring and do not show these characteristic immunofluorescent changes. Also patients with this condition develop mucous membrane lesions which are absent in this patient.

Recurrent discrete bullae, often on the legs, limited in extent and healing without atrophy were noted by Lever (1953) in association with benign mucous membrane pemphigoid. Sneddon & Church (1955) observed that some cases of bullous pemphigoid started with a localized group of blisters which persisted for several weeks before the generalized eruption, and often occurred at the site of some existing skin injury.

Lever (1965), reviewing the reports of Foldvari (1958) and Eberhartinger & Niebauer (1961), recognized localized bullous pemphigoid as an entity in which bullae tend to be on the lower legs, more commonly unilateral than bilateral. They arise on normal or erythematous skin and the resulting areas of denudation heal slowly. In some patients bullae form continuously, in others intermittently with remissions of several months. This condition occurs in elderly persons and takes a very chronic course, extending over many years, for example as long as 30 years in one of the patients of Foldvari (1958). Stewart *et al.* (1971) reported cases which had a high titre of antibasement membrane antibody, confirming the close relationship with bullous pemphigoid.

Our patient supports the view that although this condition is a definite clinical entity, it must be classified as a variant of bullous pemphigoid, which may remain localized indefinitely.

REFERENCES

EBERHARTINGER, CHR. & NIEBAUER, G. (1961) Zur Prognose und Therapie des Pemphigus Vulgaris und ähnlicher Erkrankungen. *Hautarzt*, **12**, 503.

FOLDVARI, F. (1958) Pemphigus ou pemphigoide. *Dermatologica*, **117**, 296.

LEVER, W.F. (1953) Pemphigus. *Medicine*, **32**, 1.

LEVER, W.F. (1965) In: *Pemphigus and Pemphigoid*, pp. 82–83. Charles C.Thomas. Springfield, Illinois.

SNEDDON, I.B. & CHURCH, R. (1955) Diagnosis and treatment of pemphigoid—report on 22 cases. *British Medica Journal*, **ii**, 1360.

STEWART, W.M., LAURET, PH, LECROQ, CL. & THOMINE, E. (1971) Pemphigoide bulleuse et alopecie cicatricielle, pemphigoide bulleuse et recidive *in situ*. *Bulletin de la Société française de Dermatologie et de Syphiligraphie*, **78**, 412.

(4) Porphyria cutanea tarda

DR MICHAEL KLABER, DR HARVEY BAKER AND DR A.WARIN

The London Hospital and St John's Hospital, London

J.P., male, aged 79 years. Retired house decorator. Hospital case no. 71 62 24.

History. In November 1973, a carcinoma of the colon was resected at another hospital. Postoperatively, he developed retention of urine, his acid phosphatase was slightly raised and he was found to have osteosclerotic secondary deposits. A clinical diagnosis of carcinoma of the prostate was made and he was started on ethinyloestradiol 0·02 mg three times daily. Later he developed a leuco-erythroblastic anaemia for which he received 42 units of blood and Feospan capsules once daily.

In September 1974 he began a 7 month stay in Iran. Following a day spent in the sun he developed a blistering rash on the backs of his hands together with increased skin fragility. These symptoms did not resolve on his return to this country. At this stage he was put on Honvan (stilboestrol diphosphate) 100 mg three times daily.

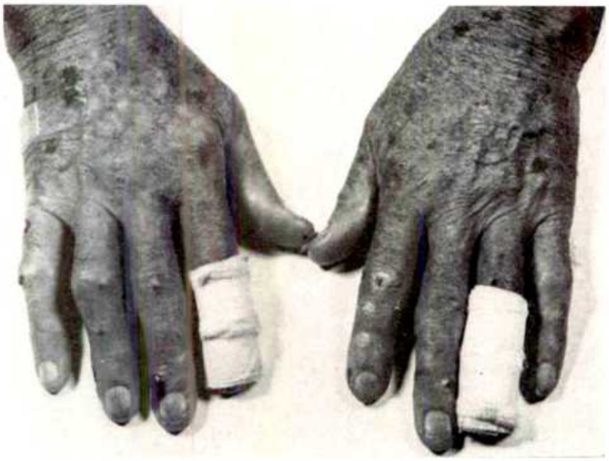


FIGURE 1. Sclerosis and bullae on hands.

He had had malaria in 1925 and 1930 and he drank four pints of beer and one whisky a day on average.

Examination (December 1975). He is deaf and has facial hypertrichosis and bullae with scarring at the sites of previous bullae on the backs of his fingers (Fig. 1).

Investigations. Hb 10·5g%, MCV 110, WBC 5800/mm^3, ESR 124/h, alkaline phosphatase 15·6 K.A. units/100 ml. Serum albumin 32 g/l, SGOT 74 units/ml, BSP retention 28% at 45 min. Creatinine clearance 43 ml/min. Plasma iron (on five occasions) 54–235 μg/100 ml. Iron binding capacity (on five occasions) 180–315 μg/100 ml. Serum ferritin 3970 μg/ml (NR 200). Serum folate 8·8 μg/l. Serum B12 940 pg/l. Acid phosphate 0·5 units/100 ml (formol stable 0·3). Chest X-ray, osteoblastic metastases ? prostatic. Liver biopsy (Dr Vernon Roberts. Fig. 2) showed increased fibrous tissue with an infiltrate of lymphocytes and occasional polymorphs in the portal tracts. There was moderate fatty change and some focal necrosis with very large amounts of iron in the hepatocytes, Kupffer cells and portal tracts. Bone marrow showed megaloblastic erythropoiesis with excessive iron stores. Monochromator testing (Prof. Magnus) showed an urticarial reaction at 400 mm. Twenty-four hour urinary porphyrins:

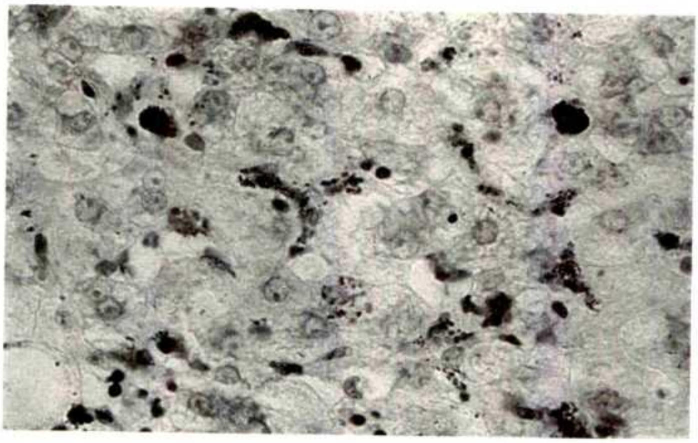


FIGURE 2. Liver biopsy.

	12.10.75	22.10.75	6.11.75	13.4.75	19.12.75	N.R.
Copro	628	179	623	545	482	< 283 μg
Uro	6941	3860	4768	3276	3008	< 41 μg

24 hour urinary iron:

5 11.75	8.11.75	12.11.75	19.12.75	N.R.
3·8	1·3	1·0	0	< 0·5 mg

Treatment. He was asked to stop drinking alcohol, and oestrogen and iron therapy were withdrawn. Desferrioxamine 500 mg i.m. daily was given from 4.11.75 for 6 weeks. The urinary iron outflow was satisfactory at first but tailed off later. The urinary porphyrins fell steadily and should be followed by improvement of his skin, initially with cessation of spontaneous blistering and later by loss of abnormal fragility.

Comment. Several aetiological factors were involved; he was on ethinyloestradiol and later stilboestrol for a presumed carcinoma of the prostate (although adequate prostatic biopsy by Prof. Blandy failed to show any evidence of malignancy). He was also suffering from gross iron overload, following the transfusion of 42 units of blood, together with oral iron therapy. Finally, he was exposed to the fierce Iranian sun. He now has abnormal monocytes in his peripheral blood and he may be developing a myelomonocytic leukaemia. Haberman, Rosenberg & Menon (1975) compared cases of porphyria cutanea tarda precipitated by alcohol and by oestrogens. Not surprisingly, the former group tended to be older men (mean age 57 years) and to have elevated serum iron and iron-binding capacity, while the latter were women of mean age 39 years in whom these values were low or normal. Otherwise there appeared to be no difference. Liver biopsy did not help in the differentiation and hepatic siderosis was an almost constant abnormality as stated by Jean, Lambertenghi & Ranzi (1968). Our patient drank moderate amounts of alcohol and there was no evidence of alcoholic hepatitis.

Venesection is clearly the treatment of choice (Ippen, 1961; Ramsay *et al.*, 1974). However, it would seem imprudent in view of his anaemia and controlled cardiac failure. Chloroquine in full dosage is hepatotoxic in PCT as well as causing retinopathy. In low dosage (125 mg twice weekly) it appears to be safe. (Kordač & Semrádová, 1974).

REFERENCES

HABERMAN, H.F., ROSENBERG, F. & MENON, I.A. (1975) Porphyria cutanea tarda: a comparison of cases precipitated by alcohol and oestrogens. *Canadian Medical Association Journal*, **113**, 7.

IPPEN, H. (1961) Allgemeinsymptome der späten Hautporphyrie als Hinweise für deren Behandlung. *Deutsche medizinische Wochenschrift*, **86**, 127.

JEAN, G., LAMBERTENGHI, G. & RANZI, T. (1968) Ultrastructure study of the liver in hepatic porphyria. *Journal of Clinical Pathology*, **21**, 501.

KORDAČ, V. & SEMRÁDOVÁ, H. (1974) The treatment of porphyria cutanea tarda with chloroquine. *British Journal of Dermatology*, **90**, 95.

RAMSAY, C.A., MAGNUS, I.A., TURNBULL, A. & BAKER, H. (1974) Treatment of porphyria cutanea tarda by venesection. *Quarterly Journal of Medicine*, **43**, 1.

(5) Late onset erythropoietic uroporphyria

DR A.MACDONALD, DR D. NICHOLSON AND DR ROGER WILLIAMS

Westhill Hospital,

E.A., male, aged 55 years. Building labourer. Hospital case no. 85 10 36.

History. The patient presented in 1975 on account of a vesicular eruption affecting the light exposed areas of the face, ears and hands which commenced in the summer of 1974, subsided spontaneously during the winter and recurred with greater severity in the summer of 1975. He also noticed increasing lethargy over the previous 4 years and the passage of red urine for 5 years. He had recently received Aldomet and Moduretic for mild hypertension.

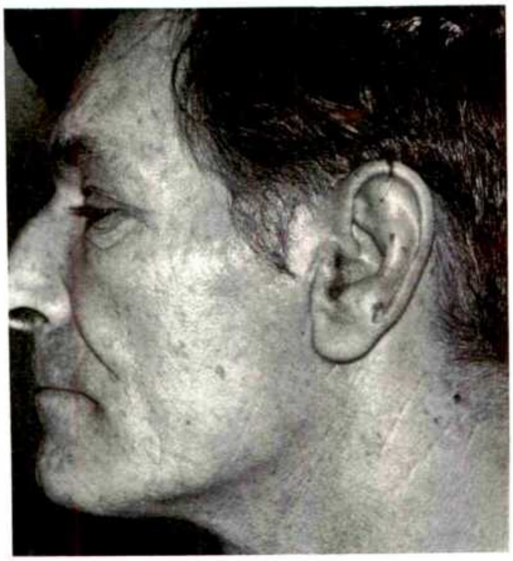


FIGURE 1. View of side of face showing crusted erosions affecting face and ears.

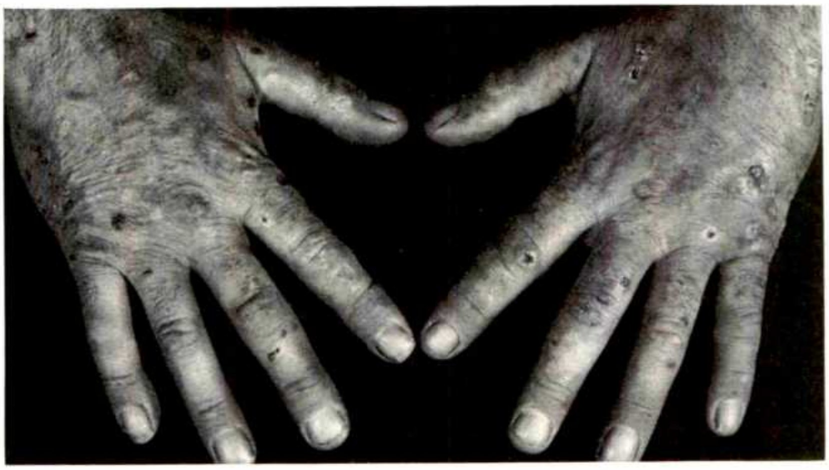


FIGURE 2. Photograph showing hyperpigmentation, crusted erosions and pitted scars on dorsa of hands due to late onset erythropoietic porphyria.

Examination. There is pitted scarring with pigmentation on the face, ears and hands (Figs 1 and 2). The liver extends 2 in. below the costal margin and the spleen is just palpable.

Abnormal investigations. Hb 9·6 g/dl, PCV 28·5%, MCV 82 fl, MCH 28·3 pg, MCHC 34·0 g/dl.

Leukocyte count 8000 cells/μl, Platelet count 85000/μl, ESR 32 mm/h.

Blood film—anisocytosis, poikilocytosis, hyperchromasia. Reticulocyte count 2·6%. Microfluorospectroscopy revealed brilliant fluorescence in less than 1% of the circulating red cells with weaker fluorescence of a larger percentage.

Bone marrow examination revealed erythroid hyperplasia and increased megakaryocytes. Microfluoroscopy revealed nuclear and cytoplasmic fluorescence of the fluoroblasts indicating the presence of uroporphyrin and possibly coproporphyrin.

Porphyrin assays. Faecal porphyrin 1292 μg/g wt of dry stool, consisting mainly of coproporphyrin I with a trace of pentacarboxylic, heptacarboxylic and uroporphyrins. No isocoproporphyrin was noted.

Urinary porphyrin 13·5–14·0 mg/day.

Composition of urinary porphyrin

Porphyrin	% of total porphyrin	Isomers present
Uroporphyrin	70·0	Wholly I
Heptacarboxyl	4·5	Almost wholly I
Hexacarboxyl	1·2	Insufficient for study
Pentacarboxyl	7·1	About 80% I
Coproporphyrin	17·2	Wholly I

Radioactive ^{51}Chromium: Tagged red cell studies revealed diminished erythrocyte survival ($T\frac{1}{2}$ = 17 days). Surface counting over the spleen showed early accumulation of radio-activity with low counts over the liver. The results are compatible with a mild haemolytic state accompanied by hypersplenism.

Normal investigations. Blood urea, electrolytes, liver function tests, serum proteins, immunoglobulins, serum iron and total iron binding capacity, haploglobin, Coombs test, serum folate and vitamin B_{12}, chest X-ray, barium swallow, lymphangiogram and coagulation screen.

Biopsies. Histology of the liver revealed mild fatty change and extramedullary haematopoiesis. Direct immunofluorescence of skin from non-light exposed area (arm) revealed positive fluorescence with IgG attached to the basement membrane and sleeving the blood vessels, characteristic of porphyria.

Treatment. The eruption at present is quiescent, and treatment consists of the topical application of a cream consisting of Dihydroxyacetone 3% + Lawsone 0·13% in a vanishing cream base. Cholestyramine one sachet daily. Carrots 1–2 lb/week.

Comment. This man presented with what looked like classical porphyria cutanea tarda due to a neoplasm or lymphoproliferative disorder affecting the liver. However, numerous investigations revealed no intrinsic disorder of the liver. The porphyrin excretion profiles and microfluorospectroscopic studies revealed findings identical to those seen in classical Gunther's disease (congenital porphyria, erythropoietic uroporphyria). The diagnosis of erythropoietic uroporphyria is further supported by the presence of red urine, photosensitivity and haemolytic anaemia.

Erythropoietic uroporphyria presenting at this late age has been reported on only two previous occasions (Kramer *et al.*, 1965; Pain *et al.*, 1975).

Genetic studies, involving measurement of urinary and red cell uroporphyrin levels, are being done on the relatives. At this stage, it is not known why the disorder expressed itself at this time nor can the possibility that this patient has an acquired disorder resembling classical EUP, but due to some metabolic derangement or extrinsic factor, be excluded.

The topical light screening agent used in this patient has been found by Taddeini & Watson (1968) to be the most effective light screening agent in this condition but β-carotene has also been of benefit in ameliorating the photosensitivity (Sneddon, 1974).

Splenectomy may help especially if the haemolytic anaemia is severe. Other treatments include infusion of Hemin (Watson *et al.*, 1974) and hypertransfusion (Seip & Erickson, 1975).

REFERENCES

KRAMER, S., VILGOEN, E., MEYER, A.M. & METZ, J. (1965) The anaemia of erythropoietic porphyria with the first description of the disease in an elderly patient. *British Journal of Haematology*, **11**, 666.

PAIN, R.W., WELCH, F.W., WOODRUFFE, A.J., HANDLEY, D.A. & LOCKWOOD, W.H. (1975) Erythropoietic uroporphyria of Gunther first presenting at 58 years with positive family studies. *British Medical Journal*, **iii**, 621.

TADDEINI, L. & WATSON, C.J. (1968) *The Clinical Porphyrias, Seminarssin Haematology*, Vol. V, No. 4, p. 342.

SNEDDON, I.B. (1974) Congenital. *Proceeding of the Royal Society of Medicine*, **67**, 593.

WATSON, C., BOSSENMAEIER, I., CARDINAL, R. *et al.* (1974) Repression by haematin of porphyrin biosynthesis in erythrocytes precursors in congenital erythropoietic porphyria. *Proceedings of the National Academy of Sciences*, **71**, 278.

ERICKSON, L. & SEIP, M. (1975) The effect of various therapeutic trials on the porphyrin excretion in a case of congenital erythropoietic porphyria. *Acta Paediatrica Scandinavica*, **64**, 287.

(6) Generalized morphoea with bullous lesions and psoriasis

DR D.A.BURNS AND DR I.SARKANY

Royal Free Hospital, London

H.M., male, aged 65 years.

History. He first developed areas of morphoea on both legs in 1973. Subsequently there was gradual spread to involve the skin of the trunk, upper limbs, face and scalp. He was treated with D-penicillamine in 1974, but therapy was discontinued after 9 months following detection of proteinuria. In October 1974 he developed a vesiculo-bullous eruption in the region of both ankles and over the dorsa of the feet. The changes on the left ankle progressed to extensive ulceration. He has also suffered from psoriasis since 1964.

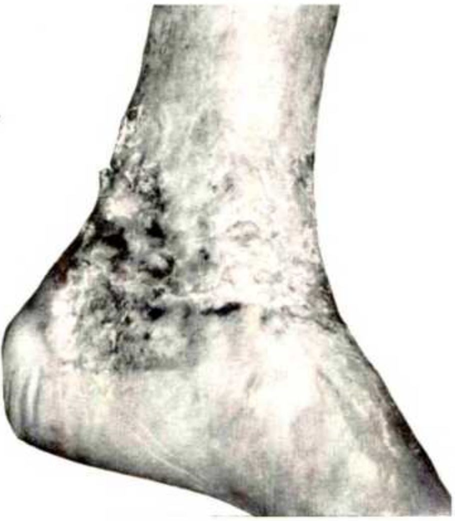


FIGURE 1. Bullous morphoea around ankle.

Examination. There is extensive morphoea involving the trunk and limbs, face and scalp. The hands are spared. There are psoriasiform areas on the elbows, lower back and legs and a vesiculo-bullous eruption over both ankles, some of the bullae being haemorrhagic (Fig. 1). Extensive ulceration is present over the outer aspect of the left ankle.

Investigations. There is no evidence of systemic involvement. Skin biopsy from a psoriasiform area on his lower back: 'The epidermis is thin and shows mild parakeratosis and mitotic activity. The dermis is sclerotic.'

Comment. This patient exhibits generalized morphoea with superimposed psoriasis. The bullous component has been described previously in patients with morphoea (Feiwel & Calnan, 1956; Calnan, 1958; Garfinkel, 1963) and histologically resembles lichen sclerosus, showing gross upper dermal oedema.

REFERENCES

CALNAN, C.D. (1958) Oedematous scleroderma. *Proceedings of the Royal Society of Medicine*, **51**, 321.

FEIWEL, M. & CALNAN, C.D. (1956) Scleroderma. *Proceedings of the Royal Society of Medicine*, **49**, 236.

GARFINKEL, H.H. (1963) Widespread morphoea. *British Journal of Dermatology*, **75**, 122.

(7) Acute widespread morphoea

DR M.P.JAMES AND PROFESSOR P.F.D.NAYLOR

St Thomas' Hospital, London

S.P., female, aged 52 years. Clerk. Hospital case no. 62 58 76.

History. The patient was well until her legs began to swell in June 1975; this was partially responsive to diuretics. She experienced intermittent, transient anterior lower leg pain and, by October 1975, stiffness and limitation of movement at the knee and elbow joints with some redness of the skin on the arms. There is no significant past or family history.

Examination. The skin on the dorsa of the feet and legs up to the thighs was hard and thickened, with some erythema of the upper thighs. There was slight pitting oedema around the ankles and similar, but less marked changes on the upper limbs, upper chest and breasts. Small blisters developed later on the lower leg. There was limited flexion and extension at ankle, elbow and knee joints, most marked in the latter. There was no demonstrable muscle weakness. General examination was normal.

Investigations. The antinuclear factor was positive 1:50. The 24 h creatine excretion was 0·7 mmol (normal 0–0·4 mmol/24 h). A persistent eosinophilia of between 847/mm^3 and 1320/mm^3 was found. The peak expiratory flow was 340 l/min (expected: 360 l/min). The F.V.C. was 2·6 l; the F.E.V. 1 was 2 l (expected: 2·1 l) and the ratio of F.E.V.1 to F.V.C. was 80%. The EMG studies (right deltoid) suggested a mild myositis or myopathy. Skin thickness of forearm: 16.10.75 *left* 1·5 mm, *right* 1·6 mm; 16.1.76 *left* 2·2 mm, *right* 2·0 mm (normal range for females under 65 years 0·9–1·4 mm).

The following investigations were normal or negative: full blood count and E.S.R.; plasma urea and electrolytes; serum calcium, phosphate and urate; plasma creatinine and creatine; liver function tests; serum immunoglobulins; creatinine clearance and urinary protein; plasma creatine phosphokinase; ECG; thyroid function; smooth muscle, mitochondrial and gastric parietal cell antibodies; chest X-ray and barium swallow; lymphangiograms.

Histology. Skin from the right calf and left breast show marked hyalinization and swelling of collagen in the mid and lower dermis which is beginning to compress the skin appendages. In places, septa of new connective tissue are extending deeply into the fat. The upper dermis is oedematous and contains a variable infiltrate, including eosinophils.

Muscle biopsies from the right calf show swollen muscle bundles separated by a round cell infiltrate (Fig. 1).

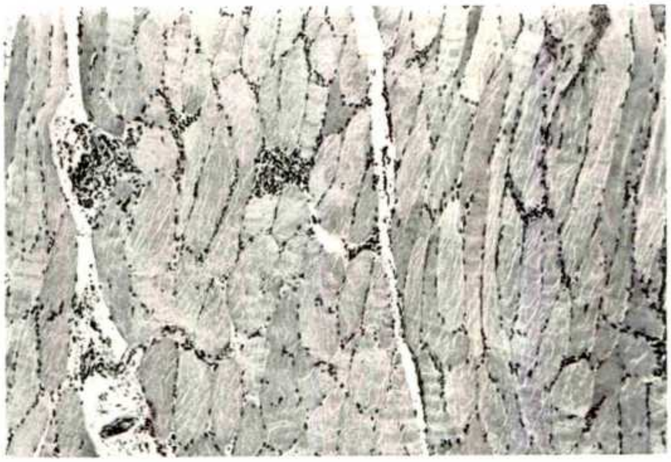


FIGURE 1. Lymphocytic infiltration in muscle biopsy.

Treatment. Anti-depressant drugs for a reactive depression. Physiotherapy for limitation of movements.

Discussion. Widespread morphoea is an uncommon condition with a good prognosis for life. Christianson *et al.* (1956) described forty-four cases lasting from 6 months to 33 years. No deaths were attributed to the disease; two subsequently developed scleroderma.

Myositis may occur under localized morphoea (Hickman & Sheils, 1964). The raised urinary creatine excretion, E.M.G. and biopsy changes suggest a significant but asymptomatic myositis in our patient.

Blistering in morphoea is rare. Garb & Sims (1959) described one case and reviewed twenty-five more. Blisters are subepidermal and seen mainly on the trunk; but legs and arms may be involved. The cause is unknown, though compression of dermal lymphatics has been suggested. The small blisters in our patient are likely to be related to the oedema accompanying the inflammatory stage of the disease.

The skin thickness was measured using a modified radiographic technique (Black, 1969). Results show an increase in forearm skin thickness during a 3-month period when the disease was clinically progressing. We suggest that this technique is a simple, objective method for following the course of sclerotic conditions, especially those involving the limbs.

REFERENCES

BLACK, M.M. (1969) A modified radiographic method for measuring skin thickness. *British Journal of Dermatology*, **81**, 661.

CHRISTIANSON, H.B., DORSEY, C.S., O'LEARY, P.A. & KIERLAND, R.R. (1956) Localised scleroderma. *Archives of Dermatology*, **74**, 629.

GARB, I. & SIMS, C.F. (1959) Scleroderma with bullous lesions. *Dermatologica*, **119**, 341.

HICKMAN, J.W. & SHEILS, W.S. (1964) Progressive facial hemiatrophy. *Archives of Internal Medicine*, **113**, 716.

(8) ? Scleredema of Buschke ? Eosinophilic fasciitis

DR D.J.ATHERTON FOR DR R.S.WELLS

Guy's Hospital, London

N.B., male, aged 49 years. Shipping officer.

History. The patient had a 'flu-like' illness in December 1974. Two weeks later he noticed a stiff, aching sensation in his legs and subsequently in his arms and anterior abdominal wall. This was accompanied by slight swelling of his ankles and wrists. He found difficulty in raising his arms to comb his hair, in lifting heavy weights and in ascending and descending stairs. Otherwise he felt reasonably well and there was no significant past medical history.

In February 1975 a 'woody feeling' was noted on palpation of the affected areas. Weakness was considered to be minimal. A diagnosis of dermatomyositis/scleroderma was considered likely. At this time he began to notice intermittent dysphagia.

Examination. Nearly all of the skin surface was involved, with the exception of that on the genitalia, hands and feet. His face and the back of his neck were very mildly affected. The epidermis looked relatively normal, although there was dimpling of the surface, particularly on the arms. The superficial part of the skin could be moved over the underlying tissue which was fixed and indurated. There was some limitation of joint movement, with contracture formation.

Investigations. Eosinophil counts between 1800 and 4000/mm^3. ESR 30–80 mm/h. Serum IgG 282×10^3 IU/l (normal 58–182) and IgM 396×10^3 IU/l (normal 50–196).

ANF positive to 1/80. Antinuclear DNA antibody binding 41% (normal 0–30%). LE cells could not be found. Creatine phosphokinase 71 IU/l. Trichinella complement fixation test positive to 1:40 (considered significant). TPHA and FTA positive, but WR, VDRL and TPI negative. Barium swallow probably normal. Other negative findings included rheumatoid factor, urinary mucopolysaccharides and stool microscopy. EMG was reported as showing mild myopathic changes. Muscle biopsy showed a mixed inflammatory cell infiltrate of the connective tissue separating the groups of muscle fibres without definite evidence of muscle cell degeneration.

A skin biopsy showed a normal epidermis, but a marked increase in dermal fibrous tissue extending deep into subcutaneous fat. There was some elastosis. The subcutaneous fat appeared normal.

Dr B.D.Lake (The Hospital for Sick Children, Great Ormond Street) examined the histochemistry of the skin and reported 'in cryostat sections there are deposits of a PAS-positive, strongly metachromatic substance between the collagen bundles in the deep dermis. This substance does not stain with Alcian Blue (pH 5·8, 0·15 M $MgCl_2$) in fixed paraffin wax embedded sections. These characteristics are consistent with the presence of a water-soluble acid mucosubstance.'

Course and management. He was given a course of thiabendazole as a therapeutic trial for trichinosis, which resulted in no improvement. In April 1975 oral prednisolone was started at an initial dose of 60 mg daily, reducing over a period of months to his current dose of 10 mg daily. There has been gradual slight improvement during this period.

Discussion. This patient presents a diagnostic problem. The evidence that he has a true myositis is minimal and Dr P.Rudge (the National Hospital) considers that he does not have polymyositis. In combination with a clinical picture compatible with SLE, these serological abnormalities, together with the high titre of ANF and the high levels of anti-DNA antibody would constitute very strong evidence in favour of this diagnosis. The clinical picture, however, is not that of SLE and we do not believe the patient has this condition. The absence of vasomotor or articular symptoms, the sparing of hands and feet and the lack of epidermal atrophy almost certainly exclude a diagnosis of scleroderma.

Many of the features of his disease are suggestive of scleredema of Buschke. The rapid onset following shortly after an acute febrile illness, the clinical appearance and symmetry of the affected areas, the histological and histochemical findings, and the tendency to gradual resolution would all be consistent with this diagnosis. In this condition the changes usually start on the back and sides of the neck and subsequently affect the face, arms, trunk and thighs (Curtis & Shulak, 1965).

In our patient the induration appeared to start on the thighs and has never involved the back of the neck or the face more than mildly. In addition, many of the haematological, biochemical and serological abnormalities are not characteristic of the condition. We believe however, that these atypical features do not necessarily exclude the diagnosis of scleredema of Buschke.

Shulman (1974) has recently reported two patients with what he considers a possible new syndrome, in which skin changes similar to those of scleredema of Buschke are combined with eosinophilia, raised ESR and hypergammaglobulinaemia. In both cases there was a distinctive thickening and chronic inflammatory infiltrate in the deep fascia. In one of the patients gradual resolution followed prolonged oral corticosteroid therapy. Similar cases have since been reported by Rodnan *et al.* (1975) and they called the condition 'Eosinophilic Fasciitis'. Although the histological examination did not include the deep fascia in our patient, the clinical findings, the eosinophilia and the hypergammaglobulinaemia would allow his provisional inclusion in this group of patients.

We suggest that scleredema of Buschke and eosinophilic fasciitis may be variants of a single disease, the former described by dermatologists, the latter by rheumatologists.

REFERENCES

CURTIS, A.C. & SHULAK, B.M. (1965) Scleredema adultorum. *Archives of Dermatology*, **92**, 526.

RODNAN, G.P., DIBARTOLOMEO, A.G., MEDSGER, T.A. JR & BARNES, E.L. JR (1975) Eosinophilic Fasciitis. *Arthritis and Rheumatism*, **18** (abstracts), 422.

SHULMAN, L.E. (1974) Diffuse fasciitis with hypergammaglobulinaemia and eosinophilia: a new syndrome? *Journal of Rheumatology*, **1**, Suppl. **1**, 82.

(9-11) Cornelia de Lange syndrome

DR D.M.MACDONALD FOR PROFESSOR M.GREAVES

St John's Hospital for Diseases of the Skin, London

(9) R.H., male, aged 2 years.

History. The patient was the second child of Indian parents. Delivery was at full term and the birth was normal. At birth he weighed 4·2 lb; he was noted to have multiple congenital deformities and an unusual low-pitched cry. Since birth there has been failure of normal growth and mental retardation.

Examination. There are multiple congenital abnormalities as follows: micromelia, oligodactyly, hirsutism, synophris, long eyelashes, low hairline, broad-bridged nose with anteverted nostrils, increased nose-lip distance, microstomia with 'carp-like' mouth, hypoplastic genitalia, hypospadias, cutis marmorata and ocular abnormalities. The physical abnormalities suggest de Lange syndrome.

(10) P.M., male, aged 12 years.

History. The patient weighed 3·5 lb at birth. Growth since then has been retarded and in addition there is severe mental subnormality. Behavioural disorder has manifested as self-mutilation of a mild form including lip-biting and scratching of the skin. Recurrent episodes of vomiting occurred in the early years of life.

Examination. There are multiple morphological abnormalities—hirsutism, synophris, long eyelashes, low hairline, broad-bridged nose with anteverted nostrils, small downturned mouth, widely-spaced teeth, low set ears, high arched palate, prominent symphysis mentis, evidence of self mutilation (Fig. 1), short tapering fingers, proximally located thumbs, simian crease, deficient epidermal ridge pattern, clinodactyly and syndactyly of 2nd and 3rd toes. The clinical appearance is that of de Lange syndrome.

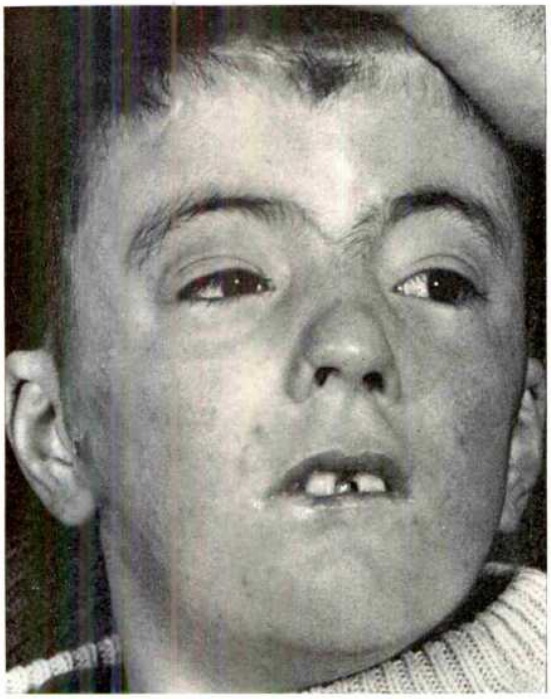


FIGURE 1. Facies: Cornelia de Lange Syndrome.

(11) A.B., female, aged 12 years.

History. The patient weighed 3·8 lb at birth. Since then there has been mild growth failure and a minimal degree of mental retardation. Dental development was delayed. A diaphragmatic hernia was diagnosed in infancy.

Examination. Multiple minor abnormalities are present. These include prominent cutis marmorata, hirsutism, synophris, long eyelashes, low hairline, anteverted nostrils, increased nose–lip distance, small downturned mouth, simian creases, deficient epidermal ridge pattern, clinodactyly and flexion deformity of fifth fingers, short fourth toes, and proximally placed thumbs. These findings are in accordance with a diagnosis of de Lange syndrome.

Comment. The syndrome first described by Cornelia de Lange (1933) comprises a group of features no less recognizable than those of Down's syndrome. Growth failure and mental retardation are usual in addition to multiple congenital morphological abnormalities. Of particular interest to the dermatologist are the hirsutism and anomalies of hair distribution (synophris, long eyelashes and low hairline often with forehead lanugo), cutis marmorata, and various deformities of the limbs—phocomelia, micromelia, oligodactyly, syndactyly and dermatoglyphic abnormalities. The syndrome was previously thought to be almost always fatal in infancy, and to be associated with severe mental deficiency and dwarfism. More recently it has become apparent that the syndrome expresses itself with variable severity and may be quite mild. No consistent biochemical, chromosomal or hereditary factors have been found to account for the syndrome (Berg *et al.*, 1970).

REFERENCES

De Lange, C. (1933) Sur un type nouveau de degeneration (Typus Amstelodamensis). *Archives de médecine des enfants*, **36**, 713.

Berg, J.N., McCreary, B.D., Ridler, M.A.C. & Smith, G.F. (1970) *de Lange Syndrome*. Pergamon Press, Oxford.

(12) The tricho-rhino-phalangeal syndrome (a family with three affected members)

DR FELIX BESSER FOR DR R.S.WELLS

The Hospital for Sick Children, Great Ormond Street, London

History. G.H., male, aged 50 years.

Mr G.H. (the father) had a series of operations on his feet beginning at the age of 16 years. He first noticed hair loss at about that time and was nearly bald by the age of 21.

His son, D.H., the proband (see pedigree), is now aged 18 years and was referred to the Orthopaedic Department at the age of 11.

His daughter, S.H., aged 15 years has also had orthopaedic operations and hair loss.

Family history. Mr G.H.'s parents are definitely unaffected, suggesting that he is the first recipient of a new abnormal mutation. The condition, as in this family, is inherited as an autosomal dominant trait.

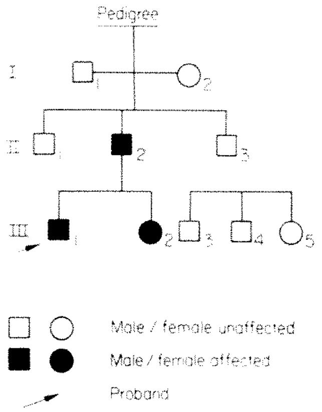


FIGURE 1. Pedigree.

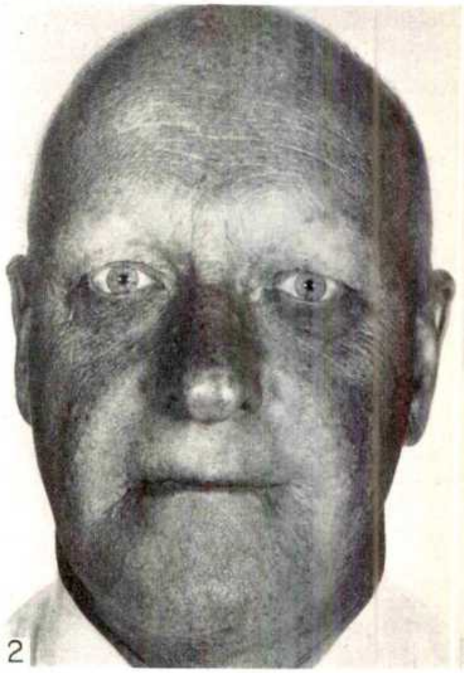


FIGURE 2. Mr G.H.

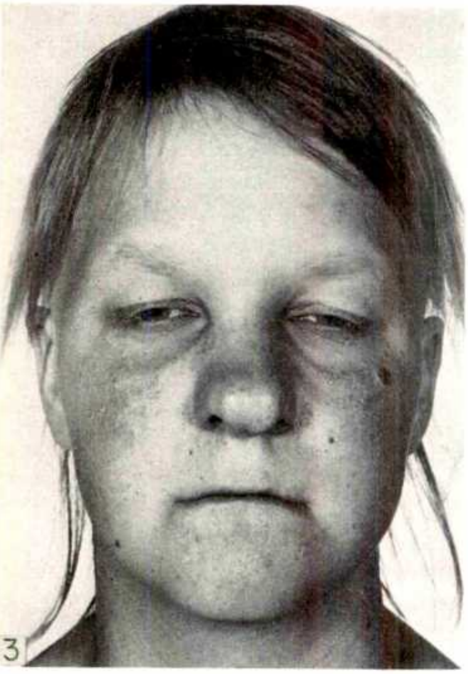


FIGURE 3. Miss S.H.

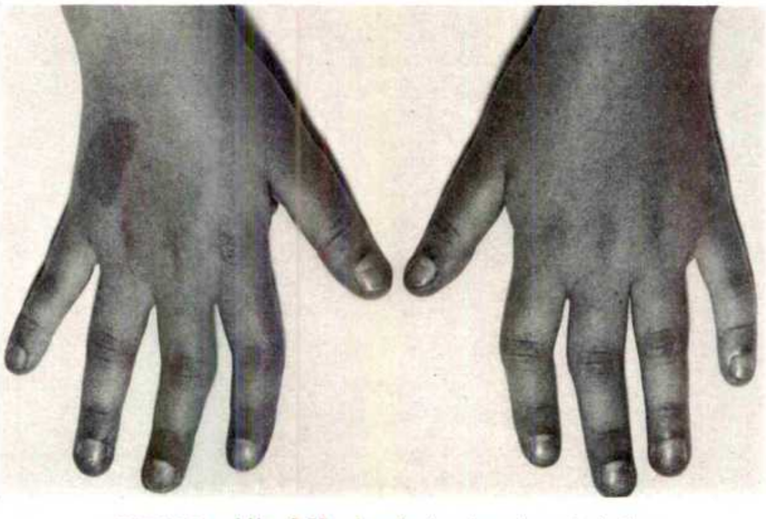


FIGURE 4. Miss S.H.—hands showing ulnar deviation.

Examination. The father has some residual fine hair, sparse eyebrows and eyelashes, and a rather bulbous, pear-shaped nose.

The son has similar fine hair, bulbous nose and some thickening of the proximal phalangeal joints. There is also some joint laxity and lateral displacement of the patellae.

The daughter has similar facial and hair characteristics and her hands show ulnar deviation at some of the proximal phalangeal joints.

Investigations. The father's hand X-rays show shortening of the middle phalanx of the left little finger. The feet show bilateral hallux valgus, valgus deformity affecting the other toes and some thickening of the shafts of the 2nd and 3rd metatarsals. The right hip shows radiological changes consistent with previous Perthe's disease.

X-rays of the son's hands show the sequelae of old coned epiphyses resulting in mal-alignment of the bones of the index and little fingers. Some residual deformities of the toes are evident. The femoral heads are small.

The daughter's hand X-rays show shortening, and characteristic deformities of the proximal ends of all middle phalanges resulting from previous coned epiphyses. There are minor changes in the proximal phalanges of both great toes. The femoral heads are small and there are changes suggestive of old Perthe's disease.

Comment. The three affected family members all show the characteristic features of the tricho-rhino-phalangeal syndrome, which was first described by Giedion (1966). In addition to the sparse hair, bulbous nose and phalangeal abnormalities this family show some additional features such as lax joints, laterally placed patellae, and small femoral heads with changes similar to those of Perthe's disease.

REFERENCE

GIEDION, A. (1966) Das Tricho-Rhino-Phalangeale Syndrom. *Helvetrica paediatrica acta*, **21**, 475.

(13-15) Juvenile dermatomyositis

DR E.B.D. HAMILTON

Department of Rheumatology, King's College Hospital

(13) A.B., female, aged 11 years. Schoolgirl.

History. January 1973. She was admitted to Sydenham Children's Hospital (Dr Upjohn) with a facial rash and periorbital oedema, as well as an erythematous rash on knuckles, elbows and knees. Her ESR was 29 mm/h, SGOT 63 units, SGPT 28 units, CPK 128 units (normal less than 50 units), LE cells negative × 2.

Dermatomyositis was diagnosed and alternate day prednisone commenced.

March 1973. There was an exacerbation of the disease with an extensive rash, oedema of her face and limb pains. Her ESR was 36 mm/h, CPK 108 units, ECG normal. Prednisone was increased to 60 mg daily, and then gradually reduced. She was discharged in April on 15 mg daily.

July 1973. She was re-admitted with severe muscle weakness, skin ulceration and joint contractures. Electromyography confirmed severe myositis, and the steroid dosage was again increased to 60 mg daily. The deformity of her knee was corrected with serial splinting.

October 1973. Hepatomegaly and abnormal liver enzyme levels were found. Liver biopsy showed extensive fatty infiltration.

October 1974. Azathioprine 50 mg daily was added to her drug regime.

March 1976. Now on prednisone 25 mg and azathioprine 50 daily, she is able to walk slowly indoors. There are slight contractures of elbows, knees and right tendo achilles. There is no calcinosis. Her CPK is 30 units.

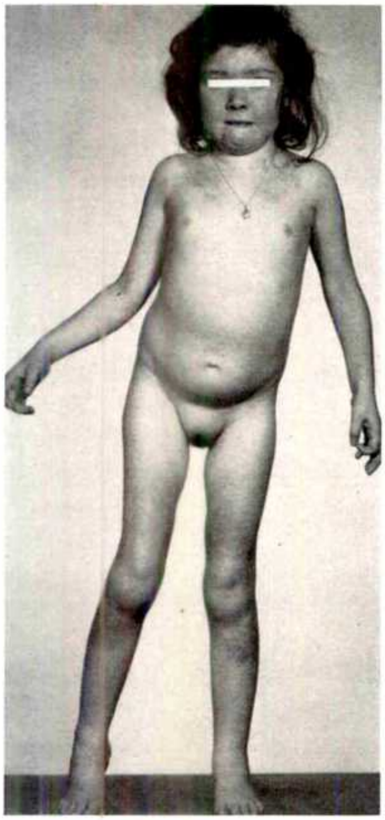


FIGURE 1. Miss A. B.

(14) A.W., male, aged 18 years

History. March 1972. He developed an erythematous rash on his face, knuckles, elbows and knees. At first he had little muscle weakness but in July an EMG showed a myopathic pattern and his CPK was 236 units. He then gradually became weaker and on 2.10.72. alternate day prednisone 20 mg was commenced.

November 1972. He was admitted to King's College Hospital with severe muscle weakness. He was unable to sit up unaided and had developed difficulty in swallowing, and contractures of elbows and knees. His ESR was 12 mm/h, ANF negative, CPK 192 units. Barium swallow showed overspill of barium into larynx and trachea. ECG showed T wave inversion which later reversed. He was treated with prednisone 40 mg daily.

1973. There was steady improvement. He returned to school and was able to play badminton on prednisone 15 mg on alternate days.

1974. It was impossible to control his disease activity on 15 mg on alternate days, especially the facial rash, and daily prednisone administration was necessary. He started work as an engineering apprentice.

1976. He still has an obvious facial rash and remains on prednisone 15 mg daily.

(15) D.H., female, aged 8 years. Schoolgirl.

History. October 1974. She developed muscle weakness and a rash on her face and hands.

December 1974. She was admitted to McMaster University Medical Centre where dermatomyositis was diagnosed. Her CPK was 1100 units. Electromyography showed a typical myopathic pattern. Prednisone 45 mg daily was started and the dose later reduced in stages. She was troubled by recurrent attacks of vomiting. There was gradual improvement in function aided by hydrotherapy.

May 1975. She was first seen at King's College Hospital. She still had a typical rash on her face and hands. Her ESR was 6 mm/h, ANF negative, CPK 60 units. An attempt was made to institute an alternate day steroid regime and by June the dosage was down to 7·5/20 mg on alternate days, but then she started to slip back.

July 1975. She was admitted with vomiting and a slight increase in disease activity.

October 1975. Prednisone was increased to 40 mg daily to control the disease. She now had marked muscle weakness and obvious joint contractures. Hydrotherapy was arranged.

November 1975. Azathioprine 50 mg daily was added for a steroid sparing effect.

March 1976. She still has a prominent facial rash but her CPK is only 20 units. She suffers from recurrent throat infections. Her prednisone dosage is 27·5 mg daily.

(16) Piebaldism

DR D.R.TAYLOR for DR T.ROBINSON

University College Hospital, London

J.H., female, aged 48 years; T.H., male, 15 years; K.H., male, 15 years; R.H., male, 5 years. Hospital case nos ED 4182/3/4.

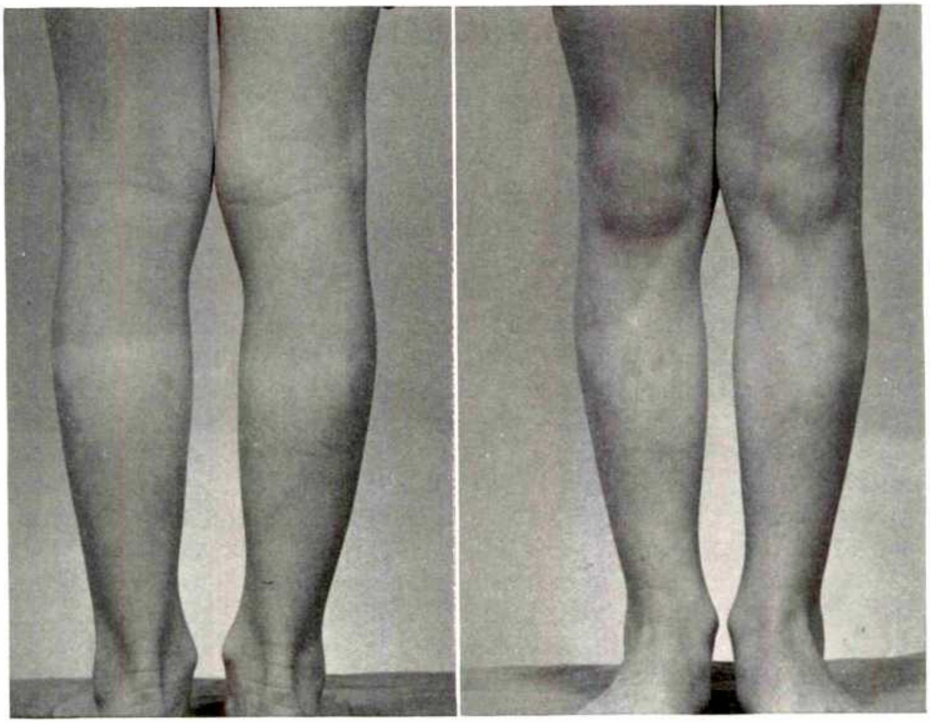


FIGURE 1. Depigmentation on the legs of R.H.

History. Mrs J.H. and her three sons give a history of depigmented patches of skin noted at birth or soon after and remaining unchanged to the present time. Other members of the family do not appear to have been affected. Mrs J.H. formerly had a white forelock, but 14 years ago developed alopecia totalis. She has an aunt who also developed alopecia areata, but there is no family history of vitiligo or related auto-immune diseases.

Examination. Mrs J.H. has a single small depigmented area on the left shin; there is now no evidence of depigmentation on the forehead or scalp. T.H. has depigmented areas on the legs, abdomen and forehead. K.H. has areas on the right lower leg with islands of normally pigmented skin within the depigmented areas. R.H. has areas on the legs and abdomen, and a triangular area on the forehead (Fig. 1). None of the sons shows a white forelock.

Comment. This family illustrates the classical clinical presentation of piebaldism with predominantly ventral distribution. Although alopecia areata is associated with vitiligo and is found in about 50% of cases of Vogt–Koyanagi syndrome, the occurrence of alopecia totalis in the case described is presumably coincidental.

(17) Lipodystrophia centrifugalis abdominalis infantilis

DR R. J. CAIRNS

St Bartholomew's Hospital, Rochester

D.B., female, aged 11 years. Schoolgirl.

History. She was first seen at the age of 2 weeks, with erythematous, rather violaceous, bizarre shaped, keloid-like areas on her abdomen, apparently present at birth. She has been followed up since then and by the age of 2 years the lesions had a definite keloidal appearance and it was apparent that new lesions were appearing. An additional feature was that the lesions caused itching. She is otherwise normal.

Examination. Bizarre pseudokeloidal patches are present on the abdomen, extending around each loin (Fig. 1). Similar patches are present over the scapulae and left gluteal region. On palpation the lesions give the impression of subcutaneous emphysema, with lack of underlying resistance. Scaliness and perilesional erythema are not marked features.

Investigations. All routine investigations have been normal. There are no skeletal abnormalities.

Biopsy. Histology of atrophic area (Dr H.Haber, 27:2:62, St John's Hospital): 'The striking feature in the corium is the absence of the appendages. The corium itself shows numerous thin-walled vessels, increased cellularity and stretching of its bundles in some places. The elastica shows perhaps fragmentation of its fibres in some places'.

Further biopsy (Dr E.Wilson Jones, 27:2:73, St John's Hospital): 'There is no inflammation or direct histological evidence of atrophy. The collagen bundles show some hint of coarseness and disorganization, so this could possibly be some form of scleroderma'.

Comment. The rather cumbersome designation might be shortened to progressive focal lipodystrophy, although this may be a primary dermal disorder. The term lipodystrophia centrifugalis abdominalis infantilis was introduced by Imamura *et al.* (1971) who described five cases of an hitherto unpublished clinical entity. This case is the first, to my knowledge, to be reported outside Japan, and shows striking similarity to Imamura's cases and to a further patient reported by Makino *et al.* (1972). The disorder appears to be primarily cutaneous and focal. The areas on the abdomen were probably

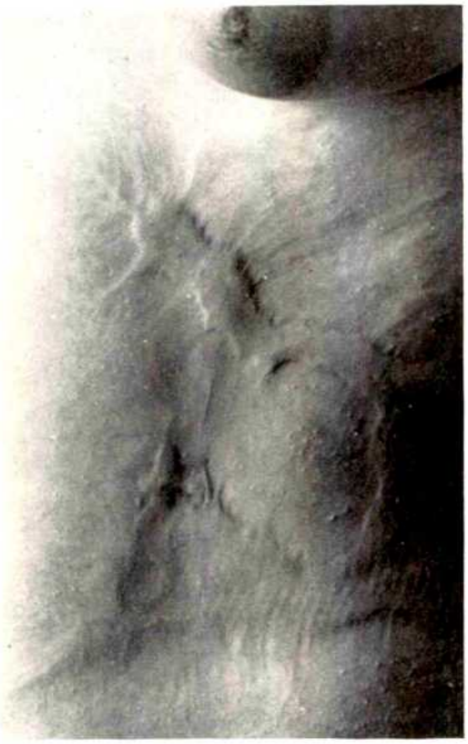


FIGURE 1. Bizarre shaped abdominal scarring.

present at birth and have since progressed. These pseudokeloidal lesions differ from those seen with aplasia cutis, macular atrophy, atrophic morphoea (Pasini-Pierini), congenital fat necrosis and Rothman–Makai syndrome. This condition differs from Gorlin's syndrome of focal dermal aplasia in that here the dermis is affected and the fat herniates through the skin. Also, in Gorlin's syndrome, other mesodermal dysplasias are found.

REFERENCES

IMAMURA, S., YAMADA, M. & IKEDA, T. (1971) Lipodystrophia centrifugalis abdominalis infantilis. *Archives of Dermatology*, **104**, 291.

MAKINO, K., INONE, T. & SHIMAO, S. (1972) Lipodystrophia centrifugalis abdominalis infantilis. *Archives of Dermatology*, **106**, 899.

(18) Lichen aureus

DR J.R.S. RENDALL FOR DR T. ROBINSON

University College Hospital, London

J.P., female, aged 11 years. Schoolgirl. Hospital case no. BE 9506.

History. In February, 1975 the patient first noticed a red area under the skin at the medial aspect of her left ankle. She assumed this to be a bruise, but did not remember any injury at that site. Subsequently, it extended up the medial aspect of the left leg and into the groin, becoming reddish-brown in colour. There was no irritation and she has remained perfectly well in all other respects.

Examination. There is a rust coloured, slightly raised lesion extending up the leg from the medial aspect of the left ankle to the groin (Fig. 1).

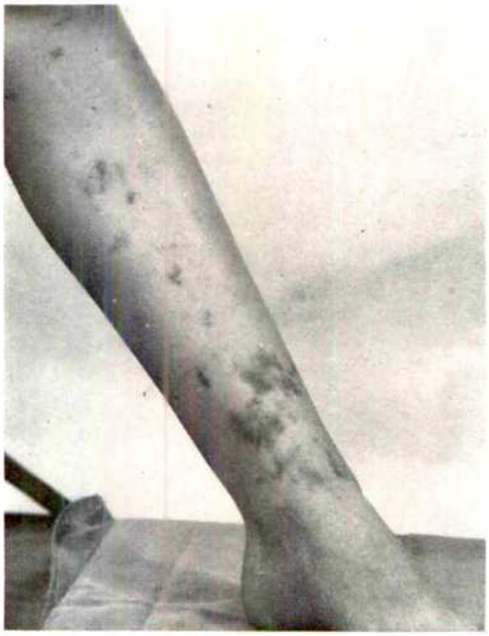


FIGURE 1. Lesion on left leg.

Investigations. Full blood count and E.S.R. both normal.

Biopsy. The epidermis is normal. There is a mixed infiltrate consisting of lymphocytes and histiocytes in the superficial dermis. Some of the cells within this infiltrate give a positive staining reaction for iron (Fig. 2).

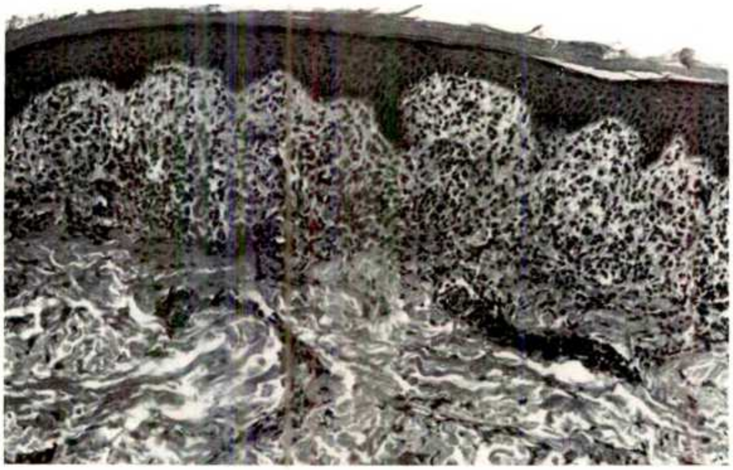


FIGURE 2. Mixed infiltrate giving a positive staining for iron (H & E, × 100).

Treatment. One section of the lesion is being treated by twice daily applications of Dermovate ointment.

(19 & 20) Xeroderma pigmentosum in brother and sister

DR MICHAEL KLABER AND DR J.S.PEGUM

The London Hospital

(19) A.G., male, aged 13 years. Schoolboy. Hospital case no. 73 96 97.

History. He was born in England of West Indian parents. Ten years ago he was seen elsewhere with 'phlyctenular conjunctivitis' which never completely settled. Three years ago he went to St Lucia and, after some months of attending an open-air school, began to develop hyper- and hypo-pigmented areas with some scaling on his face and arms. Gradually all light-exposed areas became involved. About 1 year ago he developed lesions on his nose and lip which were 'trimmed' in St Lucia. The skin condition appears to have been ascribed to eczema. His eyesight gradually deteriorated until he was almost blind. He has one affected sister and two other siblings are still in St Lucia. There is no parental consanguinity.

Examination. He is photophobic and has generalized xeroderma with profuse macular hyper- and hypo-pigmentation and numerous keratoses; only the buttocks are spared. He has squamous cell carcinomata on his lower lip, forehead and right forearm and numerous basal cell carcinomata including six on his face (Fig. 1). He has vascularization of his cornea and an epibulbar squamous cell carcinoma of the left eye.

Investigations. Hb 10·8 g/dl; Sickle cell positive (Hb A and Hb S); Serum copper 80 μg/100 ml (slightly low); Plasma and urinary amino-acids normal. Monochromator testing (Prof. Magnus): no visible reaction. Fibroblast and lymphocyte cultures to assess DNA repair in progress (Dr Gianelli)

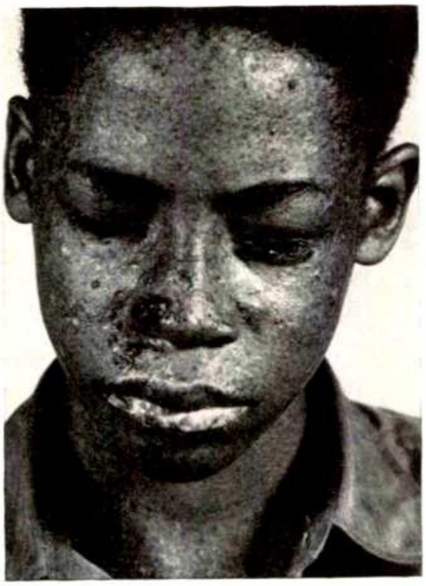


FIGURE 1. Xeroderma pigmentosum with basal and squamous carcinomas.

Histopathology showed three squamous cell carcinomata, six basal cell carcinomata and some actinic keratoses.

Treatment. Excision and grafting of many actinic malignancies was performed, together with a lamellar graft to his left eye.

(20) S.G., female aged 7 years. Sister of Case 1. Hospital case no. 73 96 98.

History. She was also born in England and went to St Lucia 3 years ago and attended an open-air school. Eighteen months ago she developed photophobia and 1 year ago identical skin lesions which in her case involved her whole body, not sparing the buttocks.

Examination. She is similar in appearance to her brother, photophobic with hypo- and hyper-pigmentation and numerous actinic keratoses. She has squamous cell carcinomata on her lower lip, nose and left shoulder. Her eyes, too, have corneal vascularization but no tumour formation.

Investigations. Hb 9·4 g/dl; sickle-cell positive (HbA and HbS). Serum copper 95 μg/ml (normal). Plasma and urinary amino-acids normal. Monochromator testing and fibroblast and lymphocyte culture as above.

Histopathology: three lesions were squamous cell carcinomata and the rest were keratoses.

Treatment. All lesions that were clinically malignant or pre-malignant were excised.

Comment. Césarini *et al.* (1975) stated that hypopigmented macules on areas of skin not exposed to sunlight are common in patients with this condition. They found few or absent melanocytes in these hypopigmented areas and suggested that there was a genetic melanocytic abnormality predisposing to increased sensitivity to ultraviolet radiation. Our cases do not support this. The girl, who wore only rather flimsy pants which allowed some light through, has many hypopigmented macules; her brother, who wore more substantial pants, has clinically normal buttock skin.

The overt malignancies have been dealt with and we are now considering treatment of the other lesions with topical 5-fluorouracil. This seems the most logical course in addition to shielding them from ultraviolet light in the future. Cryotherapy or dermabrasion have been used but do not appear to be as successful. With 5-fluorouracil, the premalignant keratoses disappear and the hyperpigmentation fades (Kint, Kint & Schuddinck, 1972; Midana & Leigheb, 1970; De Morogas & Ginenez-Camarasa, 1970).

REFERENCES

Césarini, J.P., Bioulac, P., Moreno, G. & Prunieras, M. (1975) Hypopigmented macules of sun exposed skin in xeroderma pigmentosum. An electron microscopic study. *Journal of Cutaneous Pathology*, **2**, 128.

Kint. A., Kint, R.T. & Schuddinck, L. (1972) 5 FU in the treatment of xeroderma pigmentosum. *Archives belges de dermatologie et de syphiligraphie*, **28**, 263.

Midana, A. & Leigheb, G. (1970) 5 FU ointment in the treatment of benign and malignant skin lesions. *Dermatologica*, **140**, Suppl. **1**, 75.

De Moragas, J.M. & Gimenez-Camarasa, J.M. (1970) 5 FU ointment in tumours of the skin. *Dermatologica*, **140**, Suppl. **1**, 65.

(21 & 22) Lipoid proteinosis

Dr Neil Smith for Dr K.V. Sanderson

St George's Hospital, London

(21) M.C., male, aged 8 years, and (22) K.C.,* female, aged 10 years.

History. (K.C.) Her voice was noticed to be hoarse and whisper-like following laryngitis when aged

---

* This case was first reported in the *Proceedings of the Royal Society of Medicine* (1970) **63**, 888.

12 months. Thickening of lower lip was first noticed at the age of 13 months, after laceration of her lip by her teeth following a fall. Laryngoscopy when the patient was 4 showed oedema of the vocal cords. By this time small pitted scars on the face and nodular thickening of the eyelid margins were present as well as the infiltrations of the mucous membrane of the lips. (Figs 1 and 2). Repeat laryngoscopy at the age of 8 showed nodular thickening of the anterior two-thirds of the right vocal cord.

(M.C.) At the age of 16 months he developed ulcers and blisters in his mouth which resolved spontaneously over a few weeks. This was diagnosed as herpetic stomatitis. His voice has always been hoarse, and originally it was thought that he might be copying his sister. Skin and mucous membrane lesions similar to those of his sister developed throughout childhood. Direct laryngoscopy at the age of 6 showed slight thickening of the right vocal cord.

Following minor skin trauma both children have often shown delayed healing with the production of unsightly scars. Their general health has been good and in particular neither child has had epileptiform attacks, episodes of parotitis or sun sensitivity.

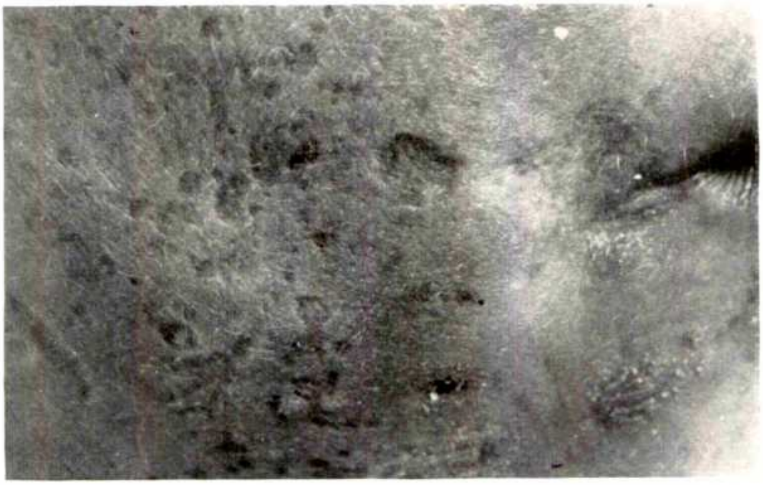


FIGURE 1. Patient M.C., depressed scars on cheek.

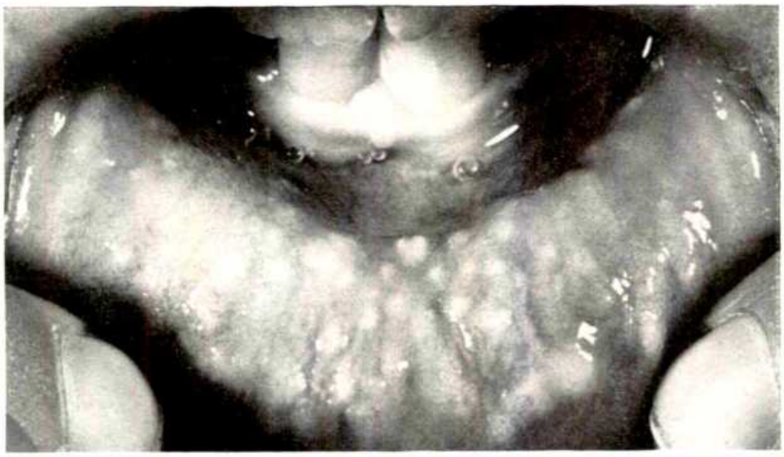


FIGURE 2. Patient M.C., infiltrations in the lower lip.

Family history. The children's parents are first cousins. No other members of the family have symptoms of the disease.

Examination. Both children show typical clinical features of lipoid proteinosis. There are pitted acneiform scars on the face, nodular thickening of the eyelid margins and infiltrations in the buccal mucosa of the lips. The voice is hoarse and whisper-like.

Investigations. Normal: Haemoglobin, W.B.C. and differential count; E.S.R.; Blood glucose; Liver function tests; Screening test for porphyrins; Serum total cholesterol; Serum triglycerides; Serum protein electrophoresis (normal apart from a raised alpha-2 globulin); Skull X-ray: normal with no evidence of parasellar calcification.

Histology. 1. (K.C.) 1969 biopsy from lip (3418/69). 2. (M.C.) 1973 Biopsy from larynx (1337/73). Both biopsies show the features of lipoid proteinosis with deposits of hyaline, eosinophilic, P.A.S. positive material in the dermis and submucosa.

REFERENCE

HOFER, PER-ÅKE (1973) Urbach-Wiethe disease. *Acta dermato-venereologica*, **53**, Supplement 71.

(23) Generalized plane xanthoma

DR M.P.JAMES AND DR S.C.GOLD

St John's Hospital, London WC2

N.T., female, aged 66 years. Retired telephone operator. Hospital case no. P 59362.

History. She has suffered from eczema since infancy, and had the occasional asthmatic attack. The eczema has sometimes been exacerbated by strong sunlight. Her brother has asthma and eczema. There is no family history of diabetes, thyroid or cardiovascular disease. Seven years ago she began to develop yellow areas on the face, and nodules on the scalp and in the ears. Myxoedema was diagnosed at Guy's Hospital 3 years ago.

Examination. She had widespread eczema involving scalp, face, upper chest and back, dorsa of hands and arms. Scattered patches of eczema were found on trunk, buttocks, legs and flexures. The eyelids and face showed widespread xanthelasma, with flat lesions on the neck, upper chest and around the ears. Both external auditory meati were partially blocked by nodular xanthomata. The scalp had soft, raised, cerebriform lesions especially over the parietal regions (Fig. 1). General examination was normal.

Investigations. The cholesterol was 8 mmol/l in February 1976 (normal 2·6–6·2 mmol/l). A persistent eosinophilia of between 11 and 24% was found. Monochromator light testing showed her to be abnormally sensitive to 'sunburn' (short UVR) irradiation. The following investigations were normal or negative: Full blood count and ESR; plasma urea and electrolytes; fasting blood sugar; fasting plasma triglyceride and cholesterol in 1973 and 1975; antinuclear factor, antimitochondrial, smooth muscle and gastric parietal cell antibodies; plasma proteins and electrophoresis; chest X-ray; urinary protein.

Histology. A nodular lesion removed from the left external auditory meatus shows foam cells and giant cells scattered throughout the dermis.

Treatment. Her recent treatment has included topical steroids to face and body; diazepam 20 mg at night; thyroxine 0·1 mg daily. Cholestyramine and nicotinic acid were prescribed in 1973, but stopped in August 1974.

Discussion. Generalized plane xanthoma is an unusual condition associated with Type III hyperlipidaemia, primary biliary cirrhosis, myeloma, dysproteinaemias and lympho-reticular neoplasms. The plasma lipids are often raised but may be normal (Slack & Borrie, 1971). There remains a group unassociated with obvious internal diseases, and with normal lipids.

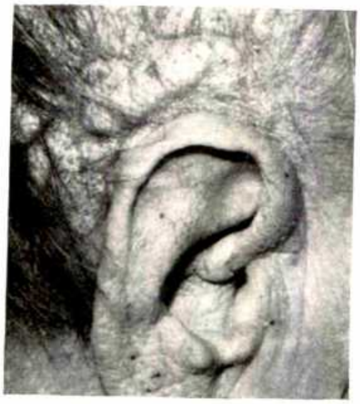


FIGURE 1. Cerebriform xanthoma in the scalp.

Walker & Sneddon (1968) described a man with longstanding atopic eczema who developed plane xanthoma on the face and upper chest. Plasma lipids were normal, but there was some increase in beta-lipoprotein. Our case is similar, but cerebriform scalp lesions and nodular lesions in the ears are unusual, and appear not to have been previously described in this condition. The plasma cholesterol was normal in 1973, but has recently been elevated. This may be related to the thyroid disease, or to a genetic tendency to hyperlipidaemia now becoming overt.

Fleischmajer, Hyman & Weidman (1964) suggested the cause might be a reactive hyperplasia of the reticulo-endothelial system with secondary xanthomatization. Hu, Ellefson & Winkelmann (1973) have shown that both normolipaemic and hyperlipaemic induced xanthomata can synthesize lipids *in vitro*. We suggest that this hyperplasia is secondary to the long standing light sensitive eczema involving chest, head and neck, possibly superimposed on a genetic tendency to lipid abnormalities.

REFERENCES

FLEISCHMAJER, R., HYMAN, A.B. & WEIDMAN, A.I. (1964) Normolipaemic plane xanthomas. *Archives of Dermatology*, **89**, 319.

HU, C., ELLEFSON, R.D. & WINKELMANN, R.K. (1973) *In vitro* lipogenesis of cutaneous xanthoma tissue. *Journal of Investigative Dermatology*, **60**, 107.

SLACK, J. & BORRIE, P. (1971) Xanthomatosis. In: *Modern Trends in Dermatology* (Ed. by P.Borrie), Vol. 4, p. 194. Butterworths, London.

WALKER, A. & SNEDDON, I.B. (1968) Skin xanthoma following erythroderma. *British Journal of Dermatology*, **80**, 580.

(24) Lichen myxoedematosus

DR J.P.H.BYRNE FOR DR R.H.MARTEN

King's College Hospital

A.W., male, aged 63 years. Bank messenger. Hospital case no. 64 74 89.

History. The patient was admitted to hospital in November, 1973 for treatment of pain in the left leg possibly due to a deep venous thrombosis. At that time the skin of his arms, trunk and face was noticed to be thickened, hyperpigmented and itchy. The infiltration of the skin had commenced in June, 1973 and has progressively worsened. Investigations for dysphagia and hoarseness revealed no abnormality. With respect to his general health, he has undergone surgery for duodenal ulceration, appendicitis and volvulus.

Examination. The skin of the arms, trunk, back and face is infiltrated, shiny and hyperpigmented. A papular infiltrate is also present on the hands, neck and retro-auricular areas (Figs 1 and 2). There is limitation of facial movements and obliteration of the normal skin creases, causing a somewhat featureless facies. There is also ankle oedema.

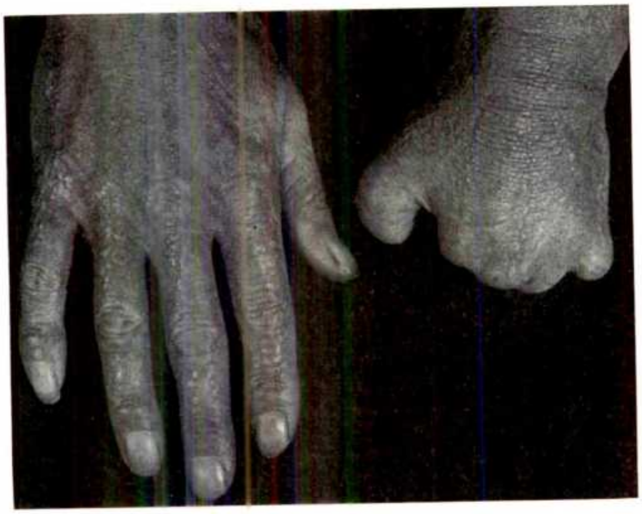


FIGURE 1. Photograph showing typical papular infiltrate of lichen myxoedematosus of the hands.

Investigations. Hb, 12·8 g/dl; WBC, 7,300 cells/μl; Platelets, ample; ESR, 15 mm/h; Protein electrophoresis, two discrete bands in the gamma region; Immunoglobulins normal; No Bence Jones protein; Immunoelectrophoresis of concentrated urine, normal; Biochemical screen, blood sugar, serum cholesterol, all normal; Auto-antibody screen negative; No LE cells; Urine glycosaminoglycan/creatinine ratio: 32 (NR for age: 15–39); ECG, Chest X-ray normal; Barium swallow, neuromuscular inco-ordination at the vallecula; Iliac crest biopsy, slight hypocellularity and iron deficiency.

Biopsy. Histology of the skin reveals focal mucinous deposits giving staining reactions of hyaluronic acid in the ground substance. There is an increase of fibroblasts in the mucinous areas and collections of pigment-containing macrophages and giant cells are present adjacent to the epidermis. The appearances are consistent with lichen myxoedematosus.

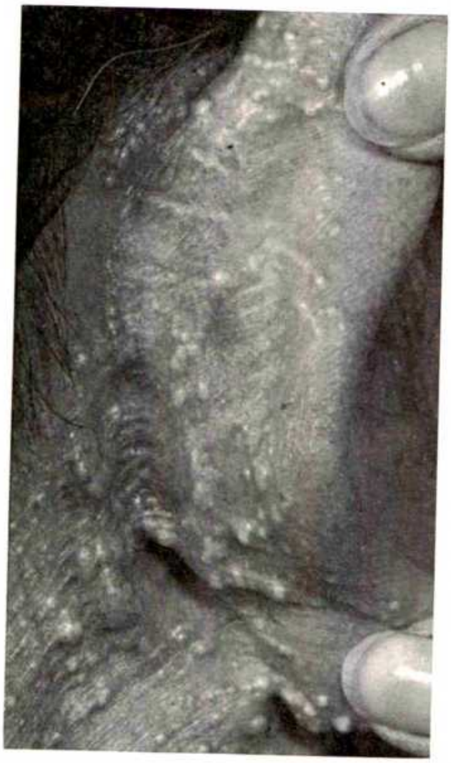


FIGURE 2. Photograph of right retro-auricular area showing heaped-up papulo-nodular infiltrate characteristic of lichen myxoedematosus.

Treatment. The patient was started on prednisolone 40 mg/day in June, 1974 but no benefit occurred. In September, he was started on a combined regime of prednisolone 40 mg/day, and melphelan 10 mg/day for 1 week repeated at 6-weekly intervals. With this treatment, the skin on the trunk and forearms became less tight but the improvement was transient and did not prevent the development of new papules occurring on the hands and neck. Over the last few months the disease has inexorably progressed and the patient is now on prednisolone 60 mg/day.

Comment. This is a typical case of lichen myxoedematosus, the severity of which is increasing in spite of treatment. The patient is edentulous and owing to the involvement of the perioral skin by the disease, it has been found impossible to insert dentures. At one stage he was considered to have a myopathy of the shoulder girdle muscles but the neurologists ascribe the muscle weakness to the tight skin. Dermatomyositis has been described in association with this disorder (Johnson *et al.*, 1973). Melphelan produced no long lasting benefit in our patient as it did in the case of Feldman *et al.* (1969). There has been a report by Howsden, Herndon & Freeman (1975) of a dramatic response of lichen myxoedematosus to the use of cyclophosphamide and it is hoped to start our patient on this treatment.

Protein electrophoresis has revealed a paraprotein in the gamma region which has not been further characterized. There is no underlying evidence of a malignant monoclonal gammopathy in this patient. The abnormal paraprotein which has been found in many cases of lichen myxoedematosus is a 7S-globulin of the G_2 type having only lambda light chains (Lawrence, Tye & Liss, 1972).

REFERENCES

JOHNSON, B.L., HOROWITZ, R.I., CHARLES, C.R. & COOPER, D.L. (1973) Dermatomyositis and lichen myxoedematosus. *Dermatologica*, **147**, 109.

FELDMAN, P.H., SHAPIRO, L., PICK, A.I. & SLATKIN, M.H. (1969) Scleromyxoedema. A dramatic response to Melphelan. *Archives of Dermatology*, **99**, 51.

HOWSDEN, S.M., HERNDON, J.H. JR & FREEMAN, R.G. (1975) Lichen myxoedematosus. *Archives of Dermatology*, **111**, 1325.

LAWRENCE, D.A., TYE, M.J. & LISS, M. (1972) Immunochemical analysis of the basic immunoglobulin in papular mucinosis. *Immunochemistry*, **9**, 41.

(25) Bisalbuminaemia

DR J.P.H.BYRNE FOR DR D.I.WILLIAMS

King's College Hospital

B.B., male, aged 33 years. Clerical worker, Royal Navy. Hospital case no. C 47628.

History. The patient presented in 1959 with a flexion deformity of the fingers of both hands, affecting mainly the little fingers but also to a lesser extent the ring, middle and index fingers. The fingers are always blue and there is no undue pallor when the hands are immersed in water. In spite of a good grip, there was an inability to extend the fingers. The family history reveals that the patient's father has similar hand deformities but is able to extend his fingers. The patient's paternal grandfather was said to have cold, blue hands.

Examination. The hands are blue and the fingers cannot be extended. There is a mosaic-like hyperkeratosis of the knuckle pads and periungual areas. The fifth little toe has been amputated and there is bilateral pes cavus. Neurological examination reveals a mixed sensory and motor neuropathy affecting the feet and hands, as manifested by diminished sensation to pinprick and light touch and minimal weakness. The patient also has a bilateral central corneal dystrophy (Schneider's).

Investigations. Hb 16·0 g/dl, WBC 7100 cells/μl, ESR 22 mm/h. Total protein 78 g/l, Albumin 45·0 g/l.

Electrophoresis reveals a distinct double band in the region of the albumin. Abnormal albumin accounts for about 50% of the total albumin. This abnormality is present in numerous relatives (Fig. 1). Immunoelectrophoresis is normal. Urinary amino acid chromatograms are normal. Cold agglutinins are positive in a titre of 1 in 16. No cryoglobulins or L.E. cells are present. Antinuclear factor is negative. X-rays of the right foot (1970) revealed osteoporosis of the distal end of the 5th metatarsal with destruction of bone and periosteal new bone formation suggestive of a localized bone infection.

A barium swallow showed loss of tone and increased calibre of the oesophagus.

Swabs from the ulcer under the right little toe have grown repeatedly *Staphylococcus aureus* and *Pseudomonas pyocyanea*. Electromyograms (1970, courtesy of Dr P.K.Thomas) revealed a peripheral neuropathy affecting motor and sensory fibres. A left sural nerve biopsy yielded insufficient tissue for diagnosis.

Other investigations performed but which are normal include W.R., Kahn, Reiter, MSU, chest X-ray, lung function tests, biochemical (SMA 12) screen, chromosome studies, lipid profile, and ECG.

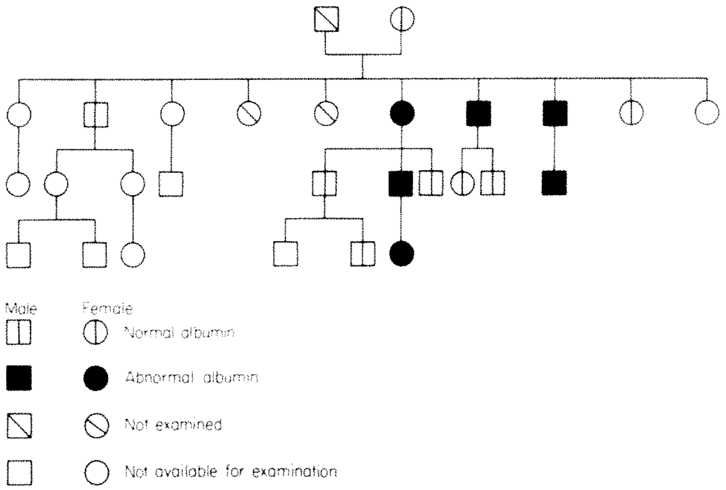


FIGURE 1. Outline of family history of bisalbuminaemia showing affected cases. Reproduced by permission of the Editor of the *Lancet*.

Biopsy. Specimen of skin from hand revealed hyperplasia of the epidermis which has an irregular saw-tooth stratum corneum and is producing abundant dense keratin. The dermis is normal. There are no specific features.

Treatment. The patient has not received any treatment aimed at ameliorating either the underlying skin condition or the protein anomaly. For 4 years he was greatly troubled by a neuropathic ulcer situated beneath the head of the right fifth metatarsal. Infection of the ulcer led to numerous hospital admissions owing to the development of lymphangitis, cellulitis and abscess formation. Eventually amputation of the little toe was needed to prevent further complications. The patient requires surgical boots.

Comment. Serum albumin variants are of two kinds. The first type is characterized by the presence of variant albumins, whose electric charge differs from that of the 'normal' albumin molecule. The second type of albumin variant is distinguished by the occurrence of albumin dimers (Bearn & Cleve, 1972). The patient shown today is an example of the first type of albumin variant, also known as bisalbuminaemia, and was the subject of a case report by Franglen *et al.* (1960). In this case, the abnormal albumin has a slower electrophoretic mobility than the normal albumin, and accounts for approximately 50% of the total albumin concentration which is characteristic of bisalbuminaemia. This anomaly is present in the same concentration in five other members of the family. The additional band in that variant of albumin synthesis characterized by dimer formation accounts for 10–15% of the total albumin. The mode of inheritance of both these albumin variants is by autosomal dominant transmission and usually the patients are in excellent health. However, the finding of similar hand deformities in the son, father and possibly grandfather suggests some relationship to the albumin anomaly. However, four other members of the family possessing the protein abnormality exhibited no dermatological disease.

REFERENCES

BEARN, A. & CLEVE, H. (1972) *Genetic variations of Plasma Proteins in Metabolic Basis of Inherited Disease* (Ed. by J.B.Stanbury, J.B.Wyngaarden and D.Fredrickson), 3rd edn, p.1630. McGraw-Hill, New York.

FRANGLEN, G., MARTIN, N.H., HARGREAVES, T., SMITH, M.J.H. & WILLIAMS, D.I. (1960) Bisalbuminaemia. A hereditary albumin abnormality. *Lancet*, **i**, 307.

(26) Sarcoidosis

Dr D.I.Vollum

Lewisham Hospital, London SE13

C.N., female, aged 38 years. Packer. Hospital case no. D 11582.

History. The patient came here from Nigeria in 1963. Early in 1974 she first noticed some nodules on her right upper arms. Over the next 18 months further lesions appeared on her face, back, arms, buttocks and thighs.

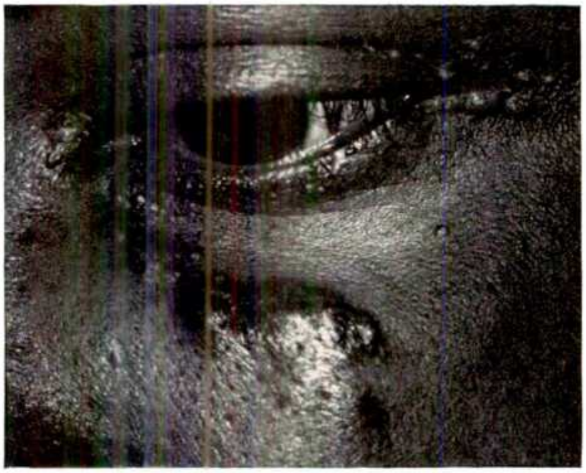


FIGURE 1. Sarcoid granuloma on left cheek.

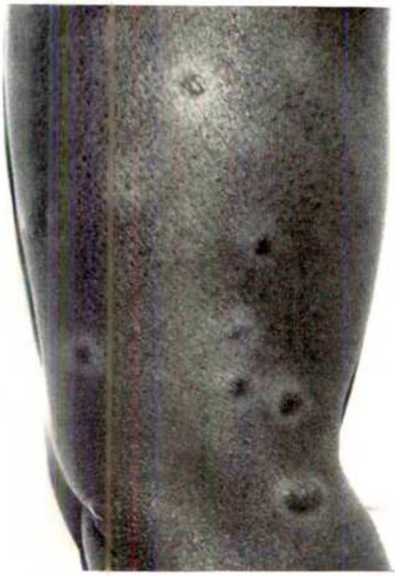


FIGURE 2. Sarcoid granulomas on right arm.

Examination. There are: small papules on both eyelids; an indurated plaque below the left eyelid with several purpuric nodules surrounding the upper border (Fig. 1); macular areas of hyperpigmentation on both ears where nodules have resolved; several violaceous nodules with surrounding hypopigmentation on her right upper arm (Fig. 2); numerous hypopigmented macules with underlying dermal nodules on left arm and thighs. Loss of left 5th toe (traumatic).

Investigations. Skin biopsy. There is a granulomatous lesion in the dermis extending into the subcutaneous tissue. There is an infiltration of histiocytes with foamy cytoplasm having a tuberculoid type of arrangement. Multinucleated giant cells are rare. The histology is compatible with sarcoidosis. Biopsy of Kveim test positive. Tine test negative. Chest X-ray July 1974: There is enlargement of the hilar and paratracheal lymph nodes. February 1976: Still some enlargement of the hilar nodes. Hand X-ray February 1976: Calcified lesions due to Loa loa. Cystic lesion head of proximal phalanx and absorption of terminal tuft right index finger.

Normal FBC, ESR, Serum Ca, PO_4 and 24 h urine for calcium.

Treatment. Since June 1975 she has been treated with chloroquin 200 mg daily, and potassium para-aminobenzoate 12 g daily.

Comment. During this period many of the lesions on the arms and legs have become smaller so that there are only macular areas of hypopigmentation where previously there were violaceous nodules. The tumour below the left eyelid and the nodules on the ears have regressed but new micropapules have appeared on the eyelids.

(27) Cutaneous ectopic schistosomiasis

DR D.M.MACDONALD AND DR J.G.L.MORRISON for PROFESSOR M.GREAVES

St John's Hospital for Diseases of the Skin, London WC2

M.G., female, aged 20 years.

History. The patient arrived in Britain from Sierra Leone 1 year ago. She first noticed itchy nodules on the abdomen in 1973. Since that time new lesions have appeared on the flanks and around the umbilicus. Since 1971 she had suffered from intermittent lower abdominal pain since a single episode of haematuria which was treated with a course of 'tablets'.

Examination. Deep seated nodules with overlying lichenification were grouped particularly around the umbilicus (Fig. 1) and in a zosteriform distribution on the flanks. There were no other abnormal physical findings.

Histology. Apparently viable eggs of *Schistosoma haematobium* with characteristic terminal spines and miracidia are seen within the dermis, associated with collections of eosinophils.

Investigations. Schistosoma complement fixation test: positive to a titre of 1 in 80. Sigmoidoscopy: normal vascular pattern, no ulceration or bilharziomata seen. Proctoscopy and rectal snips: rectal snips contain ova of *Schistosoma haematobium* which are calcified. No ova of *Schistosoma mansoni* are seen. No *Schistosoma* eggs were found in centrifuged deposits in urine. 'Sandy grains' characteristic of submucosal calcified *Schistosoma* eggs were seen on the bladder wall at cystoscopy. Biopsy of these lesions showed calcified ova.

Treatment. Niridazole (Ambilhar) 25 mg/kg/day in divided doses for 7 days is the treatment of choice. Prednisone 15 mg/day reducing sequentially is given to suppress the frequent 'allergic' complications which occur on the death of the parasites.

Comment. Cutaneous lesions of schistosomiasis caused by ectopic eggs are an extremely rare

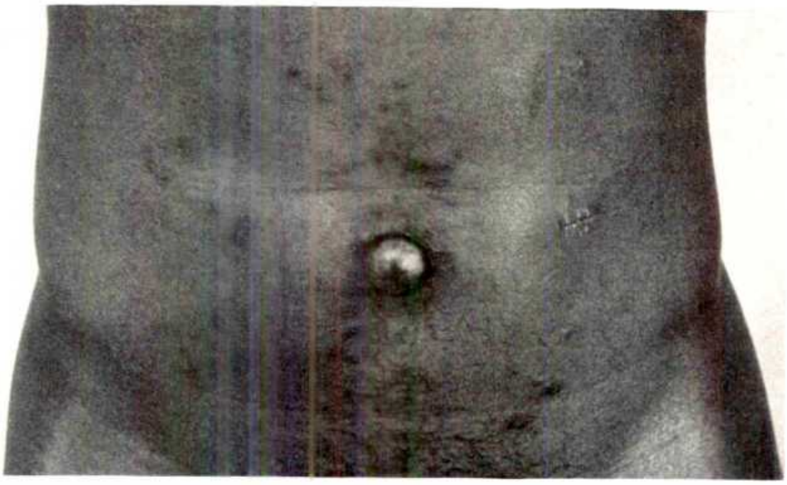


FIGURE 1. Cutaneous ectopic schistosomiasis showing the peri-umbilical distribution.

occurrence, even in endemic areas (El Mofty, 1956; El-Mofty & Cahill, 1964; Findlay & Whiting, 1971). Various routes of passage of the worms to the skin have been suggested (Saxe & Gordon, 1975; Findlay & Whiting, 1971). However we believe that the likely route in this patient is from the portal circulation via the left portal vein to the para-umbilical veins accompanying the ligamentum teres hepatis to the umbilicus where they anastomose with the superficial and deep epigastric veins of the caval system. This would account for the common distribution around the umbilicus.

REFERENCES

EL-MOFTY, A.M. (1956) Extragenital forms of cutaneous bilharziasis. *British Journal of Dermatology*, **68**, 252.

EL-MOFTY, A.M. & CAHILL, K.M. (1964) Cutaneous manifestations of schistosomiasis. *Dermatologia Tropica*, **3**, 157.

FINDLAY, G.H. & WHITING, D.A. (1971) Disseminated and zosteriform cutaneous schistosomiasis. *British Journal of Dermatology*, **85**, Supplement 7, 98.

SAXE, N. & GORDON, W. (1975) Schistosomiasis of spinal cord and skin. *South African Medical Journal*, **49**, 57.

(28) Acnitis

DR S.PEIRIS FOR DR D.I.WILLIAMS

King's College Hospital, London

D.P., male, aged 53 years. Clerk. Hospital case no. 88 89 07.

History. In October 1975, the patient developed an erythematous papulo–pustular eruption affecting the periorbital areas, cheeks and neck. There was no past history of any serious medical illness or dermatosis. As far as can be ascertained, the patient had never received B.C.G. vaccination.

Examination. Numerous reddish-brown firm papules and pustules are present on the eyelids (Fig. 1) and there are also flesh coloured papules on the anterior aspect of the neck.

Investigations. The following investigations were normal: full blood count, erythrocyte sedimentation rate and biochemical screen. A chest X-ray revealed an irregular calcified opacity surrounded

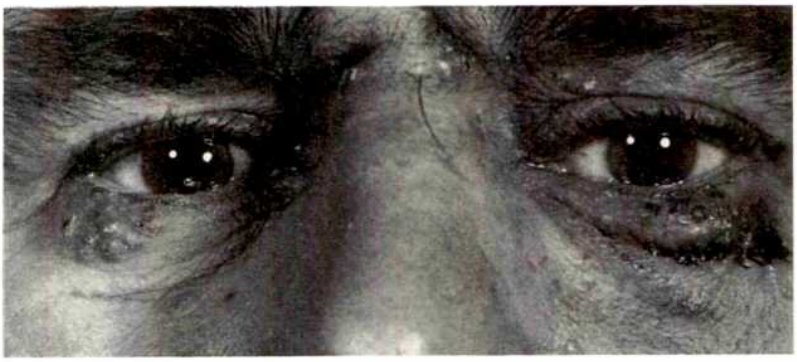


FIGURE 1. Photograph showing close-up view of papulopustular eruption of lower eyelids typical of acnitis.

by smaller shadows in the right upper zone. A radiological diagnosis of calcified tuberculoma was made and tomograms supported this.

Cultures of sputa and bronchial washings obtained at bronchoscopy were negative for *Mycobacterium tuberculosis*. The Tine test (1:1000) was positive whereas the Kveim test was negative. Cultures of swabs from the pustular lesions on the eyelids were sterile.

Histology. Biopsies of papules from the left lower eyelid and neck revealed the presence of multiple granulomata some of which showed caseation, with epithelioid cells and Langhan's giant cells. Acid fast bacilli were not seen and culture of biopsy material was negative for *M. tuberculosis*.

Treatment. The opacity in the right upper zone has been diagnosed as a calcified tuberculoma and at present the patient is not receiving anti-tuberculous treatment but is being seen at frequent intervals in case tuberculous activity occurs.

Comment. This patient has typical acnitis. Scott & Calnan (1967) reviewed the literature concerning acne agminata, a term synonymous with acnitis, and studied seventeen cases. They felt that the condition was a distinct entity unrelated to tuberculosis and should not be classified as a tuberculide, in spite of the fact that many of their cases had a positive tuberculin test.

Our patient has a positive Tine test and probably a calcified tuberculoma but it is considered that these findings are fortuitous and unrelated to the skin eruption.

REFERENCE

SCOTT, K.W. & CALNAN, C.D. (1967) Acne agminata. *Transactions of St John's Hospital Dermatological Society*, **53**, 60.

(29) Lupoid sycosis

DR J.KIRBY AND DR D.D.MUNRO

St Bartholomew's Hospital, London

B.H., male, aged 39 years. Analytical chemist. Hospital case no. 50 98 11.

History. For 5 years he has had recurrent irritating lumps over his right cheek, which resolve with scarring (Fig. 1).

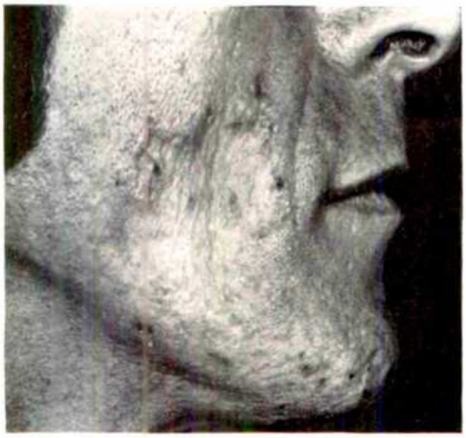


FIGURE 1. Scarring lesion on right cheek.

There is no previous history of skin disease, no acne vulgaris. His mother and sister have been similarly affected.

Examination. There are folliculo-papular lesions on his right cheek in a circular pattern occurring around a diffuse centrally scarred area (Fig. 1).

Biopsy. There are prominent pilosebaceous follicles with keratin plugging, and a chronic inflammatory reaction in the corium with a whorled fibroblastic proliferation in one area. No pustular, granulomatous or giant cell foci are seen, but some plasma cells and Russell bodies are present among the inflammatory cells.

Treatment. *July* 1973. Tabs. Deteclo 1 twice daily produced some improvement after 3 months. *Dec. 1975*. After a recurrence of his facial rash, erythromycin by mouth was prescribed with slight improvement. *Feb. 1976*. Prednisone 10 mg on alternate days was started and the erythromycin continued.

Comment. The clinical appearance and histological changes are compatible with a diagnosis of lupoid sycosis.

(30) Benign hyperglobulinaemic purpura of Waldenstrom

DR R.CLAYTON FOR DR M.FEIWEL

St Mary's Hospital, London W2

A.D., female, aged 46 years. Housewife.

History. For 10 years the patient has observed showers of slightly irritant pinpoint dark red raised lesions of the lower legs following prolonged standing and alcohol ingestion. Over the years the lower legs have become hyperpigmented (Fig. 1).

Examination. There is diffuse maculopapular hyperpigmentation of the lower legs and small fresh purpuric papules may occasionally be seen, but ulceration never occurs.

Investigations. Histology shows an acute non-specific vasculitis with perivascular polymorph and occasional chronic inflammatory cell infiltrate. No fibrinoid necrosis is seen.

Normal results: haemoglobin, blood count and film, bone marrow, urea and electrolytes, urine

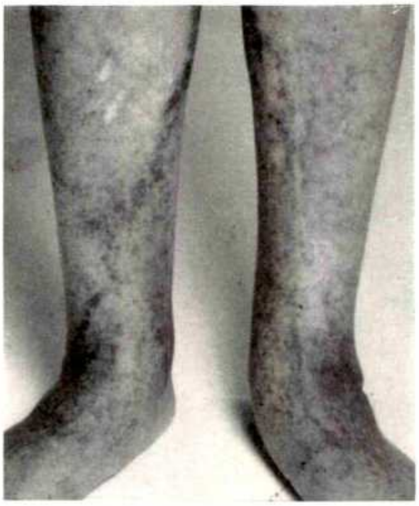


FIGURE 1. Lower legs showing maculo-papular hyperpigmentation.

microscopy, liver function tests, quantitative IgG and M, chest X-ray, auto antibody screen. No LE cells, significant DNA antibodies, cryoglobulins or urinary Bence Jones protein detected.

Abnormal results: ESR 55–110 mm/h, protein electrophoresis shows broad gammaglobulin band with marked increase of IgG precipitin and showing no obvious distortion (polyclonal), IgG 42·0 g/l (6–16). Rheumatoid factor positive, ANF positive 1:640.

Anticomplementary titre, 0·32 units left—C_1 binding complexes with large amounts of complexed IgG, total haemolytic complement activity 64%, C_4 64%, C_3 89%.

Immunofluorescence of skin lesions (Figs 2–4) shows foci of finely granular IgG and C_3 in the dermal papillae which in the case of IgA is closely related to blood vessel walls.

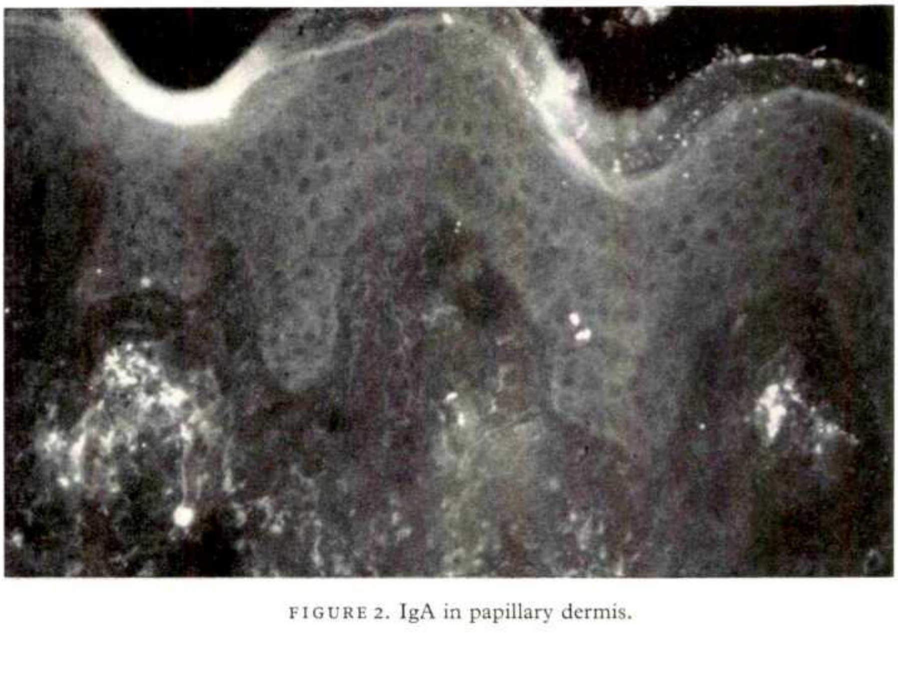


FIGURE 2. IgA in papillary dermis.

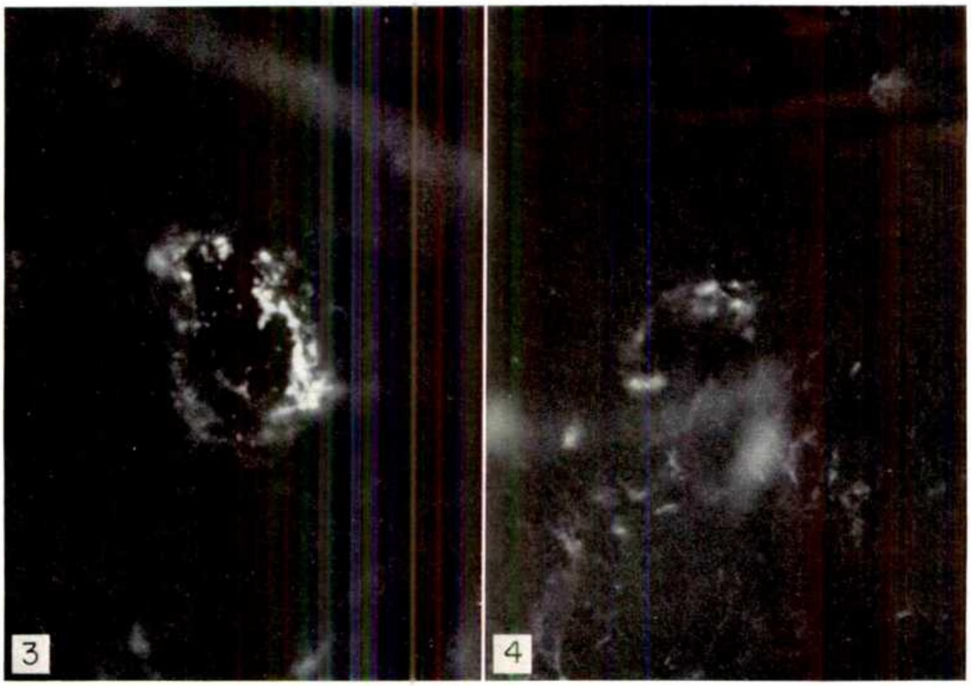


FIGURE 3. IgA in dermal papillary blood vessel.
FIGURE 4. C_3 in papillary dermis.

Comment. In recent years some of the various syndromes of cutaneous vasculitis have been shown to be associated with circulating immune complexes and immunofluorescence of the lesions has shown immunoglobulin and complement in the dermal vessels suggesting complex deposition. In this patient with Waldenstrom's benign hyperglobulinaemic purpura (WBHP) it would appear that IgA complexes as shown on immunofluorescence are deposited within dilated dermal leg vessels following prolonged standing and alcohol ingestion. These findings are similar to those in the case of Perks, Green & Gleeson (1974) although they suggested that in their case the immunologically mediated vasculitis was not mediated by the complement system because they detected no complement in the lesions and serum C_3 complement level was normal.

Patients with WBHP have been observed to develop Sjögren's syndrome, systemic lupus erythematosus and polyarthritis (Hambrick, 1958) suggesting that the condition belongs to the spectrum of auto-immune disease. This patient has both IgG antibodies (rheumatoid factor) and antinuclear antibodies.

ACKNOWLEDGMENTS

The immunofluorescence was done by Mr Gerald Haffenden. The photographs are by permission of Dr P.N.Cardew, Department of Audiovisual Communication, St Mary's Hospital Medical School.

REFERENCES

HAMBRICK, G.W. (1958) Dysproteinemic purpura of the hyperglobulinemic type. Clinical features and differential diagnosis. *Archives of Dermatology*, 77, 23.

PERKS, W.H., GREEN, F. & GLEESON, M.H. (1974) A case of purpura hyperglobulinaemia of Waldenstrom studied by skin immunofluorescence. *British Journal of Dermatology*, **91**, 563.

(31) Livedo reticularis with thrombocythaemia in association with polycythaemia rubra vera

DR JACQUELINE CLARKE AND DR G.C.WELLS

St Thomas' Hospital, London

V.N., female, aged 52 years. Laundress. Hospital case no. 30 14 88.

History. For 4 years this lady has had painful chilblain-like lesions on the toes and lateral borders of both feet. The condition is worse in winter and has been associated with shedding of the toe nails. There has been no response to the peripheral vasodilator isoxuprine.

Past history. She has been mildly hypertensive since 1971 but is well controlled on Navidrex K one tablet daily.

Examination. There was livedo reticularis on both ankles and buttocks, with cyanosis of the lateral border of the right foot (Fig. 1). She had superficial ulceration on the first and fifth toes of each foot. General examination was normal, including good peripheral pulses and no splenomegaly.

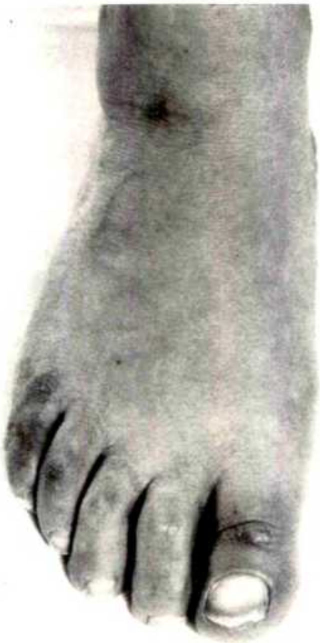


FIGURE 1. Cyanosis of the lateral border of the right foot.

Initial investigations (October 1975). Hb, 7·6 g/dl; PCV 0·31 (normal range 0·36–0·47); MCV 59 fl (normal range 75–95) MCHC 24 g/dl. (32–36) WBC $12·3 \times 10^9$/l (normal differential); Leukocyte alkaline phosphatase 176 (normal range 15–90); Platelets 1048×10^9/l (normal range $150–400 \times 10^9$/l); Platelet function tests showed reduced aggregation against ADP; Serum iron 2·6 nmol/l (normal range 14–29); TIBC 100 nmol/l (normal range 45–72).

Bone marrow (before treatment): this was hypercellular, with an increased number of megakaryocytes. There was no stainable iron.

Red cell mass (after treatment) 1960 ml (expected normal 1178–1459 ml).

Normal or negative findings: urea, electrolytes, liver function tests, uric acid, Australia antigen, cold agglutinins, cryoglobulins, immunoglobulins, ANF, barium studies.

Course and treatment. Her iron deficiency anaemia and thrombocythaemia were treated with busulphan and ferrous gluconate. She has shown haematological improvement (Fig. 2) but there has been little change in the livedo reticularis and painful toe ulceration.

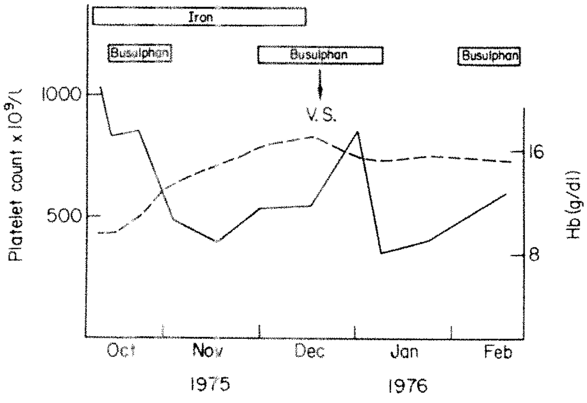


FIGURE 2. Haematological response to treatment. —— Platelets; - - - haemoglobin; V.S. venesection.

Comment. In primary thrombocythaemia, one of the myeloproliferative disorders, (Wetherley-Mein, 1974) there is an increase in circulating platelets, some of which are morphologically and functionally abnormal. The more common presentation is with spontaneous or post-traumatic haemorrhage. This is thought to be due to impaired platelet function. Decreased platelet thromboplastic activity and decreased adhesiveness have been observed (Spaet, 1969). It is also suggested that the presence of an increased concentration of platelets may impair their effectiveness (Wetherley-Mein, 1974).

Less commonly, patients present with microvascular occlusive syndromes due to platelet emboli. These have been directly observed in the retinal arteries of such patients (Singer, 1969). Copeman (1975) suggests that similar arteriolar occlusion is the mechanism of production of livedo reticularis in thrombocythaemia. The association between the two was first described by Champion & Rook (1963) whose three patients all responded symptomatically to treatment.

Our patient presented with both occlusive vascular disease and spontaneous haemorrhage, presumably from the gastrointestinal tract, as no other cause could be found for her severe iron deficiency anaemia. An initial diagnosis of primary thrombocythaemia was made. However, the rapid response of her anaemia to treatment suggested that she might have true polycythaemia rubra vera. This diagnosis has now been confirmed by the finding of a raised leukocyte alkaline phosphatase and a high red cell mass.

REFERENCES

CHAMPION, R.H. & ROOK, A. (1963) Idiopathic thrombocythaemia—cutaneous manifestations. *Archives of Dermatology*, **87**, 302.

COPEMAN, P.W.M. (1975) Livedo reticularis. Signs in the skin of disturbances of blood viscosity and blood flow. *British Journal of Dermatology*, **93**, 519.

SINGER, G. (1969) Migrating emboli of retinal arteries in thrombocythaemia. *British Journal of Ophthalmology*, **53**, 279.

SPAET, T.H. *et al.* (1969) Defective platelets in thrombocythaemia. *Archives of Internal Medicine*, **124**, 135.

WETHERLEY-MEIN, G. (1974) *Blood and its Disorders*. p. 1155. Blackwell Scientific Publications, Oxford.

(32) Painful bruising syndrome with vasculitis treated by plasmapheresis

DR C.KENNEDY, DR K.V.SANDERSON AND DR C.SPRY

St George's Hospital, and the Royal Postgraduate Medical School, Hammersmith Hospital, London

V.M., female, aged 38 years. Housewife. Hospital case no. G.73001.

History. Since 1964 she has had recurrent episodes of tender lumps in the skin, often associated with acute dyspnoea. Less frequently she has had haemoptysis, dysphagia and abdominal pain with melaena.

The skin lesions have usually started as tender erythematous oedema, becoming purpuric and sometimes ulcerating. Individual lesions last upwards of several days. They vary in size from 2 to 20 cm across, occurring particularly on the legs, trunk and arms but no part of the skin surface has been spared (Fig. 1). Similar lesions have been seen by indirect laryngoscopy in the pharynx and larynx.

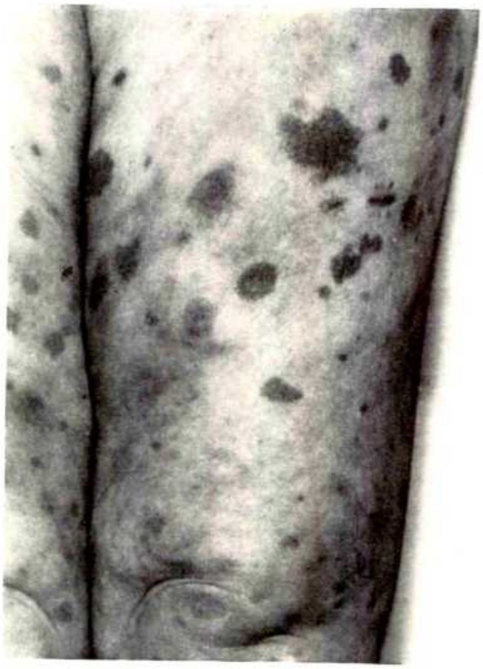


FIGURE 1. Purpuric lesions on legs.

She has otherwise been in good health, and had 5 normal pregnancies. She had a tonsillectomy at the age of 15. There is no relevant family history. She has been consistently psychiatrically stable.

Histology of skin lesions on several occasions has consistently shown a leukocytoclastic angiitis, with fibrinoid change in small blood vessels at all depths in the dermis and subcutaneous fat.

Investigations. Numerous attempts to identify a cause have been unrewarding. (a) Haematological: haemoglobin often low (iron deficiency or normochromic normocytic); white cell count, platelet count, ESR, clotting screen, fibrinolytic activity and viscosity all normal.

(b) Biochemical and Serological. The following are normal or negative: liver function tests, protein electrophoresis, immunoglobulins, cryoglobulins, cold agglutinins, syphilis serology, DNA antibodies, Coombs' test, Australia antigen, Total haemolytic complement, C 3, C 1 esterase inhibitor, ASO titre, complement fixation tests to brucella and filaria.

(c) Microbiological: no significant growth from skin, stools or urine. Mantoux test positive at 1:10,000. Virology of skin biopsy negative.

(d) Radiological: chest X-ray at various times has shown linear shadows consistent with resolving alveolar haemorrhages. Barium swallow showed soft tissue swellings during an episode of dysphagia.

(e) Immunofluorescence (direct) of the skin biopsy, including very early lesions, negative.

(f) Functional immunological tests: intradermal tests to trichophytin, candidin and bacterial antigens were negative. Similarly intradermal injections of the patient's red cells, polymorphs, lymphocytes and whole of Sephadex-fractionated serum also failed to reproduce the lesions.

Management. This patient's episodes of severe airways obstruction have partially responded to parenteral antihistamine, hydrocortisone and adrenalin.

However until recently the general course of her disease has been essentially unaffected by any of the following (in chronological order): antihistamines, antituberculous chemotherapy, antibiotics, dental clearance, corticosteroids, dapsone, azathioprine, phenformin and ethyloestrenol, dye-and preservative-free diet and Intal.

During the past year there has been a dramatic reduction of new lesions by the periodic use of plasmapheresis (Dr C.Spry).

Comment. The evolution and appearance of this lady's lesions justify the designation 'Painful bruising syndrome' (Gardner & Diamond, 1955). However she has had more severe internal involvement (especially the respiratory tract) than is usual in this syndrome (Hersle & Mobacken, 1969). She is psychiatrically stable, the lesions are not reproducible by injection of red cells, and histologically her lesions show vasculitis. This contrasts with the mild degree of inflammation usually reported (Ratnoff & Agle, 1968).

The finding of a necrotising leukocytoclastic angiitis has prompted the search for potential triggers, such as immune complexes and infection, or enhancing factors such as abnormalities of fibrinolysis (Ryan, 1974). But no clues have been found in this case.

Recently Verrier Jones *et al.* (1976) described a case of necrotising vasculitis in which circulating immune complexes were not detected by conventional methods, but intradermal injection of a serum fraction eluting between the main IgG and IgM peaks reproduced the skin lesions. This patient also responded to plasmapheresis, albeit temporarily.

It is satisfying that after a long and often life-threatening illness the patient presented here, with the features of the painful bruising syndrome and underlying vasculitis, has shown such a dramatic response to plasmapheresis. The pathogenesis of her disease is nonetheless still obscure.

REFERENCES

GARDNER, F.H. & DIAMOND, L.K. (1955) Auto-erythrocyte sensitisation: a form of purpura producing painful bruising following autosensitisation to red blood cells in certain women. *Blood*, **10**, 675.

HERSLE, K. & MOBACKEN, H. (1969) Auto-erythrocyte sensitisation syndrome (painful bruising syndrome). Report of two cases and review of the literature. *British Journal of Dermatology*, **81**, 574.

RATNOFF, O.D. & AGLE, D.P. (1968) Psychogenic purpura: a re-evaluation of the syndrome of auto-erythrocyte sensitisation. *Medicine (Balt.)*, **47**, 475.

RYAN, T.J. (1974) Vasculitis. Chapter 29, pp. 1007–1039. In: *The Physiology and Pathophysiology of the Skin* (Ed. by A.Jarrett), Vol. 3, chapt. 29, pp. 1007–1039. Academic Press, New York.

VERRIER JONES, J., CUMMING, R.H., ASPLIN, C.M., HARMAN, R.R.M. & TRIBE, C.R. (1976) Necrotising vasculitis: a circulating immune complex producing inflammatory skin lesions. *British Journal of Dermatology*, **94**, 123.

(33) Pyoderma gangrenosum with cellular immunity deficit treated with plasmaphoresis and leukocyte transfusion

DR R.CLAYTON FOR DR M.FEIWEL AND DR H.VALDIMARSSON

St Mary's Hospital, London W2

L.D., male, aged 72 years. Retired catering manager.

History. He presented 12 months ago with an ulcerated 'carbuncle' of his back present for 6 weeks. He had been on a small dose of prednisolone for 12 years for intrinsic asthma and was then on 8 mg daily. Three months later he developed similar ulcers in his left groin and on his upper abdomen. Six months later he developed similar ulcers on the right side of his forehead and left external auditory meatus.

Examination. The ulcers are irregular in outline with a ragged overhanging purplish edge and red oedematous base. There is some central epithelialization of the ulcer on his back (Fig. 1). General examination reveals no significant abnormality.

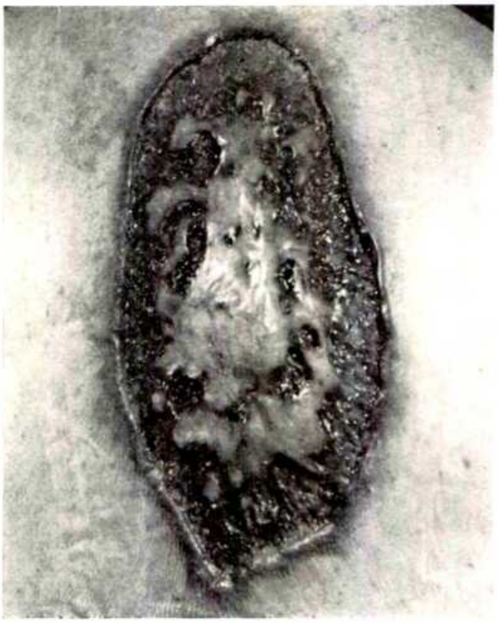


FIGURE 1. Pyoderma gangrenosum—extensive back ulceration, with early central epithelialization.

Investigations. Histology shows venous thrombosis with haemorrhage and necrosis and a polymorph and chronic inflammatory cell infiltrate. There is no evidence of tumour or granuloma.

Normal results: haemoglobin, red cell morphology, ESR, urea, electrolytes, liver function tests, protein strip, quantitative immunoglobulins, chest X-ray, barium meal and enema, sigmoidoscopy. WR & rheumatoid factor negative. No LE cells seen. No urinary Bence Jones proteins detected.

Cellular immunological studies (Dr H.Valdimarsson)

	Patient	Control	Normal range
Lymphocytes (per µl blood)	590	1490	1500–3500
T cells (%)	60	73	64–86
T cells (per µl blood)	354	1088	1300–3000
B cells (%)	18	16	10–25
B cells (per µl blood)	106	238	150–725
T/B ratio	3·3	4·8	3–8

The patient is lymphopenic and his T lymphocyte count is very low.

Lymphocyte transformation (thymidine incorporation assay)

	Counts per min per 5 ml blood phytohaemagglutinin (µg/ml culture)			Stimulation index		
				Pokeweed	PPD	
	0·1	1·0	10·0	1/10	1/100	
Patient	188	536	372	101	66	< 1·0
Control	3652	6123	3569	5013	409	12·0

The response to PHA is greatly impaired and there is no response to PWM and PPD. Also significant blocking of normal lymphocyte transformation by patients serum has been detected.

Lymphocytotoxic serum factor (^{51}Cr release assay)

Serum dilution	Patient CPM%kill	Control CPM%kill	Serum pool CPM%kill
1/2	3677 10	2467 − 12	2962 − 3·0
1/4	3123 1	2527 − 11	2478 − 11
1/8	3163 1	2333 − 14	3072 0

The difference between the patient and controls is probably not significant.

Treatment. Initially the prednisolone was increased to 30 mg daily but the further ulcers broke out as mentioned and the ulcer on his back enlarged although some central epithelialization was observed.

Comment. This is a case of pyoderma gangrenosium unassociated with ulcerative colitis, rheumatoid arthritis, chronic suppuration, hypogammaglobulinaemia or paraproteinaemia. In view of the cellular immunological studies (to be completed) it has been decided to treat this patient with plasmaphoresis. If there is no response he will be treated with plasmaphoresis and compatible leukocyte transfusion. The results will be reported.

ACKNOWLEDGMENT

The photograph is by permission of Dr P.N.Cardew, Department of Audiovisual Communication, St Mary's Hospital Medical School.

(34) Pyoderma gangrenosum, hypoplastic anaemia, tabes dorsalis

DR D.I.VOLLUM

Lewisham Hospital

H., male, aged 62 years. Retired civil servant. Hospital case no. E 88392.

History. In February 1975 he was found to have aleukaemic leukaemia. In September 1975 he bruised his left leg. The whole lower leg became oedematous and he developed a raw haemorrhagic area at the site of the bruise. Over the next 3 weeks this almost healed but in October 1975 it broke down to form an ulcer extending from the lateral side of the left knee to the lateral malleolus. The ulcer was 6 in. across and had a purulent sloughing base and a ragged bluish red overhanging border. He had a swinging fever and there was no response to a course of Pyopen. In November treatment was started with prednisone 30 mg daily, the fever settled and the ulcer gradually healed over the next 6 weeks. Recovery was complicated by a left hemiparesis of unknown aetiology. The anaemia responded to oxymetholone 100 mg daily.

Examination. There is a tissue paper scar on the lateral side of the left lower leg extending from just below the knee to the lateral malleolus (Fig. 1). The patient has a steroid facies, Argyll Robertson pupils and a high stepping gait.

Investigations. February 1975: Hb, 6·6 g%; PCV 22%; Total WBC 1300/mm^3; Platelets 44,000/

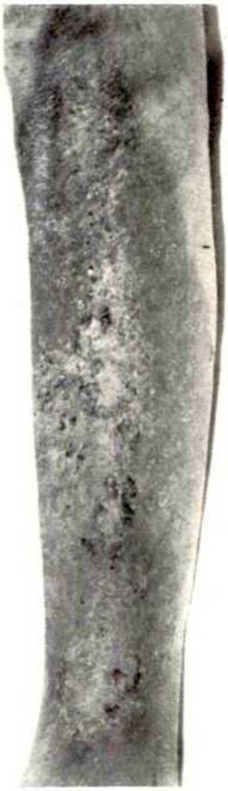


FIGURE 1. Scarring on left lower leg.

mm^3; Marrow hypoplastic. February 1976: Hb 11 g%; PCV 32%; Total WBC 1600/mm^3; Platelets 31,000/mm^3.

Biopsy from the ulcer 6.11.75: The epidermis appears pale staining and slightly oedematous with an infiltration of polymorphs. The dermis and subcutaneous tissue are congested and show an extensive diffuse inflammatory reaction with numerous polymorphs. The histology is compatible with pyoderma gangrenosum.

Comment. There have been several reports of pyoderma gangrenosum in patients with leukaemia. So far this patient has only a hypoplastic anaemia but it may be that he will eventually develop overt leukaemia.

REFERENCE

PERRY, H.O. & WINKELMANN, R.K. (1972) Bullous pyoderma gangrenosum and leukemia. *Archives of Dermatology*, **106,** 901.

(35) Chronic intravascular coagulopathy with? pyoderma gangrenosum

DR R.C.D.STAUGHTON FOR DR P.W.M.COPEMAN

Westminster Hospital, London

P.M., female, aged 68 years.

History. Ulceration developed on her right flank 6 years ago whilst she was under treatment for recurrent abscesses of her abdominal wound.

Ten months after routine cholecystectomy at St George's Hospital (in 1967), a tender mass appeared at the site of her wound. Emergency surgical exploration revealed a large extra-peritoneal abscess and *staphylococcus aureus* was cultured. Over the ensuing months a further wound abscess developed and formal drainage was performed on two occasions. During this period a 'craggy indurated ulcer' appeared lateral to the involved area. Biopsy revealed a granulomatous infiltrate, but no evidence either of foreign bodies or of any micro-organisms. Various antibiotics, metronidazole (Flagyl) and a 6-week course of Lugol's iodine and intramuscular penicillin proved unsuccessful.

After 3 years (1972) a split skin graft was applied but failed to take. Moreover the donor area on the right thigh broke down to produce an exactly similar, but larger, granulomatous ulcer. A further graft from left to right thigh was unsuccessful. On this occasion the donor area healed.

The ulcers have changed little over the years, showing no tendency to resolve spontaneously or to respond to the many forms of treatment tried.

On Examination. She shows an incisional hernia with several persistently draining sinuses, and indurated ulcers with violaceous undermined edges on her right flank (15 × 10 cm) and right thigh (30 × 25 cm).

Investigations giving abnormal results

Haematology. Chronic anaemia (Hb 9·5 g/100 ml). Film, schistocytes. Reticulocyte count 4–6%.

Fibrin degradation products, 40 μg/ml (normal < 10 μg/ml). Clot weight fibrinogen, 0·49 g/100 ml (normal 0·2–0·4 g). Platelet aggregation time, 137 s (normal 23–47 s).

Comment. The slightly increased fibrinogen level, the raised FDPs and the significantly prolonged platelet aggregation time are compatible with a state of *chronic intravascular coagulation.* This finding is borne out by the presence of schistocytes on the peripheral blood film (Dr J.P.Hayes).

ESR, 50–30 mm/h; Rose Waaler, positive 1 in 128; Latex RA, positive 1 in 64; Skin biopsies, 1972 biopsy from ulcer on right flank, and 1975: There is epidermal ulceration with an underlying infiltrate of polymorphs, lymphocytes, plasma cells and histiocytes. In the dermis granulomata are present consisting of epithelioid cells, lymphocytes, plasma cells and giant cells. There is thrombus present in arteries—within the dermis. No foreign material can be demonstrated in the sections. Stains for bacteria, acid fast bacilli, fungi and protozoa have failed to demonstrate any organisms.

Rectal mucosa. While no evidence of active inflammation is seen a minor degree of glandular shortening and distortion suggest the possibility of resolved inflammatory bowel disease (Dr A.C.Branfoot).

Investigations yielding normal results

Haematology. White count (average 6000 mm^3). Polymorph function studies, nylon column dye test, leukocyte chemotaxis.

Immunology. T Cells. Mantoux +ve 1 in 1000. Mixed lymphocyte reaction. PHA stimulation. Candida killing.

B Cells. Normal protein electrophoresis. No Bence Jones proteinuria. No immune complexes found (ultra-centrifuge analysis). No complement activation.

Biochemistry. Liver function tests; renal function tests; calcium; glucose.

Gastrointestinal tract. Barium meal and follow through; barium enema; sigmoidoscopy.

Others. Chest X-ray.

The chronicity and appearance of these ulcers suggests a diagnosis of pyoderma gangrenosum, although the markedly granulomatous histology is a little unusual.

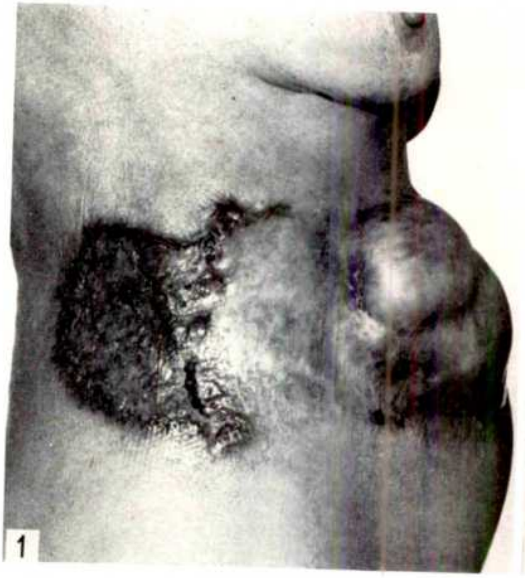
Dermatitis artefacta was considered and she was often observed disturbing her dressings. Yet the outline and size of the ulcers has remained remarkably constant (well shown by serial photographs) and no healing took place during two separate weeks of plaster of Paris occlusion.

Diligent investigation by the bacteriology departments of both St George's and Westminster Hospitals has failed to reveal any causative micro-organisms. Anti-infective measures have all been fruitless, including a recent 6-week course of rifampicin and isoniazid.

Immunological investigation shows no apparent defect of cell mediated or humoral immunity. Polymorph function is also normal.

Pyoderma gangrenosum is certainly not a distinct clinico-pathological entity. Forty percent of cases have underlying ulcerative colitis (Perry & Brunsting, 1957); other associated conditions described are rheumatoid arthritis, Crohn's disease (Stathers *et al.*, 1967) and paraproteinaemia (Cream, 1971). This lady has no symptoms to suggest any such conditions. One intriguing finding, of possible significance, is a minor degree of glandular shortening and distortion of the mucosa taken randomly from a normal looking colon. Although not specific, this is the common histological 'foot-print' of previous inflammatory bowel disease.

Whilst investigating her persistent anaemia, a mild chronic intravascular coagulopathy has been found. This has not previously been reported with pyoderma gangrenosum. Our present hypothesis is that this may be due to her blood passing through the disturbed vascular bed of these ulcers. In this context it is interesting that thrombus can be seen within subcutaneous arterioles in the 1972 biopsy. Certainly extensive angiomata may cause a similar picture (Kasabach-Merritt syndrome). A trial of aspirin and dipyndamole has just been commenced.



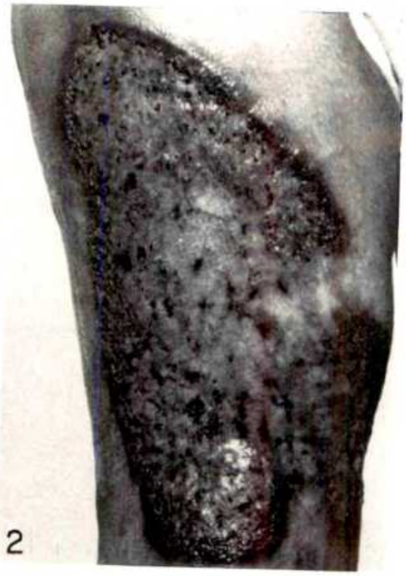


FIGURE 1. Pyoderma gangrenosum lesion on abdomen.
FIGURE 2. Pyoderma gangrenosum lesion on right thigh, the donor site for grafting.

REFERENCES

PERRY, H.O. & BRUNSTING, L.A. (1957) Pyoderma gangrenosum. *Archives of Dermatology*, **75**, 380.

STATHERS, G.M., ABBOTT, L.G. & MCGUINESS, A.E. (1967) Pyoderma gangrenosum in association with regional enteritis. *Archives of Dermatology*, **95**, 375.

CREAM, J.J. (1971) Pyoderma gangrenosum with a monoclonal IgM red cell agglomerating factor. *British Journal of Dermatology*, **84**, 223.

(36) Chronic granulomatous disease

DR J.A.CARRUTHERS AND PROFESSOR M.W.GREAVES

St John's Hospital for Diseases of the Skin, London WC2

Miss N.B., aged 8 years.

History and examination. This girl was well apart from chronic ear infection until January 1974 when she had a 'flu-like' illness. Consolidation and an effusion in her right chest suggested a diagnosis of pneumonia for which she was treated with various antibiotics, without improvement. Meanwhile her condition was worsening: she developed arthritis of her knees and wrists, erythema nodosum-like lesions on her legs, and otitis media. No causative organism could be cultured even from bronchoscopy specimens and her Mantoux test was persistently negative. After a month, during which the consolidation progressed, anti-tuberculous chemotherapy with streptomycin, ethambutol, isoniazid and pyridoxin was begun. Rapidly her fever remitted, the chest signs improved and her weight and haemoglobin rose. Approximately 1 month later the patient developed a flexural pustular rash which responded to topical therapy. She continued to improve and was well at follow-up visits.

In March 1975 the pustular rash reappeared, now unresponsive to any treatment including topical and systemic antibiotics and topical corticosteroids. In June 1975 she was first seen at St John's Hospital when she was cheerful, overweight and generally well. There was a severe pyoderma of the flexures and marked striae in these areas. This was accompanied by lymphadenopathy of axillae and groins.

Investigations. Haemoglobin, platelets and differential white cell counts were normal: her ESR varied from 32 to 45 mm/h (Westergren) and serum proteins, immunoglobulins and complement C_3 were normal. A number of plasma cortisols and 24 h urinary 17-oxo- and oxogenic steroids were normal. Bacterial cultures of the pustules grew mainly micrococci and occasionally *S. aureus* and *Strep. faecalis*. Biopsy showed suppuration centred on a damaged follicle.

White cell function tests (Institute of Child Health)

1. Nitroblue tetrazolium test (% + ve polymorphs)

	Unstimulated	Stimulated (with *E. coli* endotoxin)
Patient	0	0 No NBT reduction
Control	0	22
Father	6	40
Mother	0	54
Control	0	46

2. Incubating polymorphs with staphylococci (no. of viable bacteria)

	20 min	140 min	140/20
Patient	$1{\cdot}01 \times 10^5$	$5{\cdot}96 \times 10^4$	0·59
Control	$9{\cdot}46 \times 10^4$	$9{\cdot}06 \times 10^3$	0·09
i.e. Phagocytosis effective: moderate bacterial killing defect			
Father	$7{\cdot}16 \times 10^4$	$1{\cdot}04 \times 10^4$	0·15
Mother	$4{\cdot}46 \times 10^4$	$1{\cdot}17 \times 10^4$	0·26
Control	$6{\cdot}16 \times 10^4$	$7{\cdot}88 \times 10^3$	0·12
i.e. Probable maternal bacterial killing defect			

No abnormality of polymorph chemotaxis, opsonization or phagocytosis was found. PHA transformation of lymphocytes was also normal.

Comment. This young girl had a number of puzzling infections before the diagnosis was made. Her history highlights the importance of careful assessment of granulocyte function in addition to other tests of resistance to infection in patients with a history of chronic relapsing skin and other infections. Her first illness in 1974 was presumed to be tuberculous after the response to antituberculous chemotherapy, despite the negative Mantoux test. In retrospect it is more likely that the combination therapy was effective against a more banal infection. Similarly, her pyoderma was also responsive to an appropriate antibiotic (cotrimoxazole).

The white cell function tests demonstrate that the defect was in the intracellular killing of bacteria, all other tests being normal. It is interesting to note that her mother also has a minor bacterial killing defect, although she has no history or family history of recurrent infections.

Chronic granulomatous disease was first described only in males and an X-linked recessive inheritance has been proposed. This patient and others suggest either that the inheritance is more complex or more likely, that a number of different cellular defects may be expressed as a bacterial killing defect.

REFERENCE

HOLMES, B., QUIE, P.G., WINDHORSD, D.B. & GOOD, R.A. (1966) Fatal granulomatous disease of Childhood. *Lancet*, **ii**, 1225.

(37) Erythroderma and vasculitis

DR D.I.VOLLUM

Lewisham Hospital

M.C., male, aged 27 years. Draughtsman and fitter. Hospital case no. G 5372.

History. When aged 5 he developed a generally red skin after an attack of measles. The redness has gradually become more pronounced and the skin is always dry and scaly.

In November 1975 he developed generalized exfoliative dermatitis which seemed to start after using Boroxo detergent on his arms. There was little response to a 2-week course of Synacthen depot 1 mg on alternate days or to prednisone 60 mg daily for 2 weeks. During the latter period he developed crops of purpuric macules which crusted and ulcerated. The exfoliation, fever and vasculitic lesions cleared as the steroids were tailed off in mid January. He then developed a staphylococcal septicaemia which was treated with Orbenin and the exfoliation recurred. There was generalized oozing from the skin, the trunk was raw and there was a purulent soggy intertrigo of the flexures. Treatment with azathioprine 200 mg and prednisone 60 mg daily was started on 31:1:76. There was a slow response to treatment and recovery was complicated by a deep venous thrombosis, pulmonary embolus and chest infection.

Examination in March 1976. There is erythroderma (Fig. 1) with telangiectasia in all areas but no atrophy. Brown macular areas of hyperpigmentation are found, most marked in the axillae and on the legs. He has scaling in his scalp and atrophic scars at the sites of previous ulcers (Fig. 2).

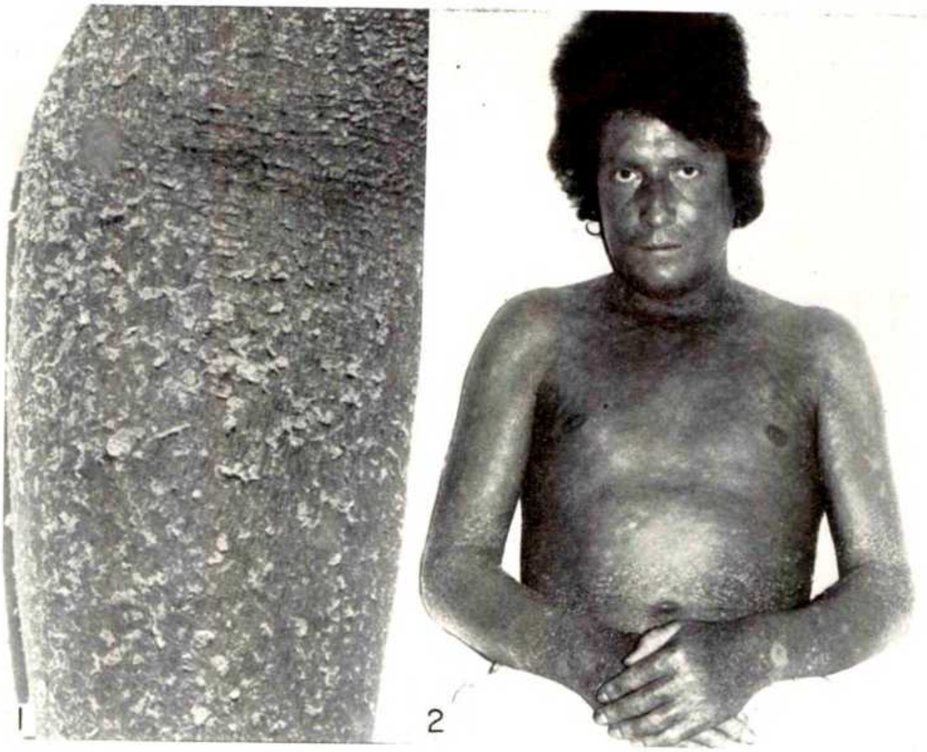
Investigations. Normal or negative: FBC, ESR, serum proteins, immunoglobulins, antinuclear factor, cryoglobulins, cold agglutinins, VDRL, LFT, Xylose test, MSSU and CXR.

Histology. Biopsy left upper arm. The epidermis appears relatively normal. There is some slight hyperkeratosis but no atrophy. There is a moderate infiltration by lymphocytes and mononuclear cells in the papillary dermis, where the capillaries appear dilated with some endothelial swelling.

Biopsy left upper leg shows focal areas of necrosis of the epidermis and papillary dermis which are essentially infarcts. The vessels show thickening of the walls due to proliferation of cells in places. The lumina are occluded. There is no fibrinoid necrosis. There is a considerable inflammatory reaction with polymorphs and some nuclear dust. Dr G.C.Wells comments that it seems to be an erythroderma with small necrotic ulcers resembling ecthyma or possibly pityriasis lichenoides.

Gland biopsy, Dublin 1973. Dermopathic lymphadenitis.

Comment. It has been suggested that this is a premycotic erythroderma.



FIGURES 1 and 2. Erythroderma with scars of previous ulcers.

(38) Sézary syndrome

DR R.C.D.STAUGHTON FOR DR P.D.SAMMAN AND DR D.E.SHARVILL

Westminster Hospital, London

D.P., male, aged 47 years.

History. He has had increasing pruritus and reddening of the skin for 3½ years, possibly exacerbated by Kenalog injections for hay fever early in 1975.

Past history. He has had hay fever for 20 years with attacks precipitated by 'cutting Lonicera hedge'.

On examination. There is a widespread, scaly, erythroderma with marked dermal oedema, particularly on his lower abdomen and upper thighs. He has mobile 'rubbery' lymphadenopathy of his groin, axillary and supra-clavicular nodes. There is hepatomegaly, no ectropion or nail dystrophy; he has male pattern alopecia.

Investigations. Sézary cells (large variety, 12–13 μm) were found in blood, lymph nodes and skin, but not in the bone marrow. White blood count $4{\cdot}9 \times 10^9$/l. Polymorphs, 51%; eosinophils, 1%; monocytes, 7%; lymphocytes, 17%; Sézary cells, 24%.

Lymphocyte studies. Sézary cells *and* normal lymphocytes 2009/mm^3 (1200–3500). T cells forming rosettes with sheep RBC 55% 1105/mm^3 (470–1800). B cells bearing IgG, IgA, IgM 26% 522/mm^3 (100–1000). Null cells 19% 372/mm^3. H rosettes with human Group O Rh neg RBC 56%.

Chromosomes. Orcein preparation. 100 cells counted.

No. chromosomes	Percentage of cells
44	1
44	2
45	1
46	90
86	3
88	2
89	1

i.e. 6% Tetraploid and pseudoploid cells.

Reticulo-endothelial system. Palpable lymphadenopathy, groins, axillae, and supraclavicular nodes. Scan, no splenomegaly. Lymphangiography, no signs of lymphoma. Lymph node biopsy: The nodal architecture is preserved but there is expansion of the paracortical (T cell) area by a cellular infiltrate consisting of histiocytes and lymphoid cells including Sézary cells and large irregular lymphoid cells. Similar lymphoid cells mixed with histiocytes are present in the sinusoids. Focal aggregates of pigment-containing macrophages are seen in some areas associated with a histiocytic proliferation. Lymphoid follicles are reduced in number but where present show very active germinal centres. Another reactive feature is the prominence of the post capillary venules.

Liver. Scan, hepatomegaly, uniform uptake. Liver function tests normal.

Kidney. Raised blood urea. (Still under investigation.) Normal creatinine clearance. Normal architecture on pyelography. No proteinuria. MSU normal.

Skin. The skin biopsy shows a focal sometimes dense infiltrate in the upper dermis composed of lymphoid cells which include the irregular (convoluted) Sézary/Lutzner type. There is only minimal infiltration into the overlying intact epidermis.

Histochemistry. Sézary type of cells show a weak positive reaction for acid phosphatase and quite often the reaction is negative; the same applies to B-glucuronidases. There is no reaction to carboxyl-esterases.

Discussion. This is a classical case of early Sézary syndrome. The laboratory findings are characteristic. Of particular interest is the normal T cell function at this stage, and the large number of cells forming H rosettes with human O negative red cells. This latter finding is thought to be correlated with a high level of 'stimulated' T cells.

Stimulant used	Plasma used	Increment (counts/min)	Index (increase/background)
Phytohaemagglutinin (PHA)	Autologous	18,100	63
Candida immunogen	Autologous	5000	13
Blocked allogeneic cells (one-way) mixed lymphocyte reaction (MLR)	Autologous	56,200	145

Normal response.

(39) Bathing trunk naevus with neoplastic transformation

Dr D.D.Munro

St Bartholomew's Hospital, London

R.W., female, aged 22 years. State enrolled nurse. Hospital case no. 51 76 73.

History. An extensive bathing trunk naevus has been present since birth. In addition many hundreds of hairy moles are present on the limbs and trunk, and the scalp has rugose changes from naevi. Multiple excisions have been undertaken when nodules occur in the naevus.

December 1974. A hard lump developed in the left loin with enlarged glands in the groin.

Examination. There is an extensive naevus; some areas are typical moles others have a neurofibroma element. Scalp has a cutis verticis gyrata appearance due to the naevus (Fig. 1).

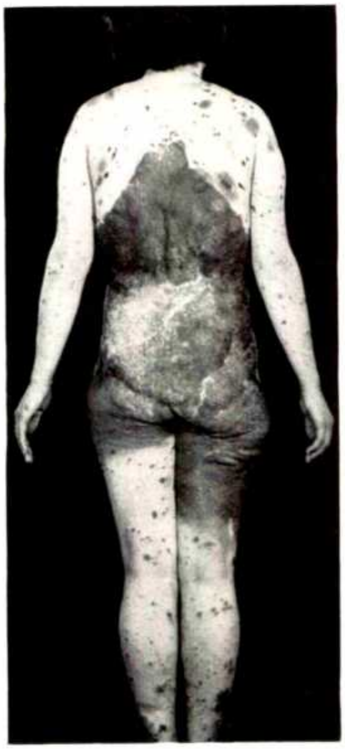


FIGURE 1 Bathing trunk naevus.

Investigations. Normal: chest X-ray; liver function tests; liver scan; full blood count.

Biopsy. Nodules from right groin and right buttock show neurofibromata with melanin pigmentation. The lump from the left loin shows a malignant melanoma in the subcutaneous tissues; the overlying naevus cell tumour shows no evidence of malignant transformation. Lymph gland from the right inguinal region revealed metastatic tumour with pigmented spindle shaped cells and scanty mitoses.

Treatment. 1. Local excision of scalp, and sections of the trunk naevus on many occasions. 2. Local excision of tumour. 3. Chemotherapy with vincristine and BCNU (1,3-bis-2-chloroethyl-1-nitrosourea).

Comment. Bathing trunk naevi are particularly prone to become malignant (Pack & Davis, 1961; Reed *et al*., 1965). This patient shows the mixed neurofibroma features, with a cutis verticis gyrata (Gross & Carter, 1967) appearance of the scalp.

REFERENCES

PACK, G.T. & DAVIS, J. (1961) Nevus giganticus pigmentosus with malignant transformation. *Surgery*, **49**, 347.

REED, W.B., BECKER, S.W. SR, BECKER, S.W. JR & NICKEL, W.R. (1965) Giant pigmented nevi, melanoma and leptomeningeal melanocytosis. *Archives of Dermatology*, **91**, 100.

GROSS, P.R. & CARTER, D.M. (1967) Malignant melanoma arising in a giant cerebriform nevus. *Archives of Dermatology* **96**, 536.

(40) Multiple keratoacanthomas

Dr D.J. VOLLUM

Lewisham Hospital

McA., female, aged 60 years. Hospital case no. E 69681.

History. For 2½ years she has had some dozens of tumours on the face, arms and trunk associated with intractable pruritus. If left the lesions tend to regress spontaneously after a few months. Recently she has developed crops of lesions on the legs. There is no family history.

Examination. On her face there are numerous pitted and some crateriform scars. There are ulcerating tumours on each cheek and multiple shiny red conical papules up to 0·5 cm in diameter on the forehead. Her trunk shows numerous hypopigmented scars. On her arms, thighs and legs, are grouped red papules up to 0·5 cm in diameter.

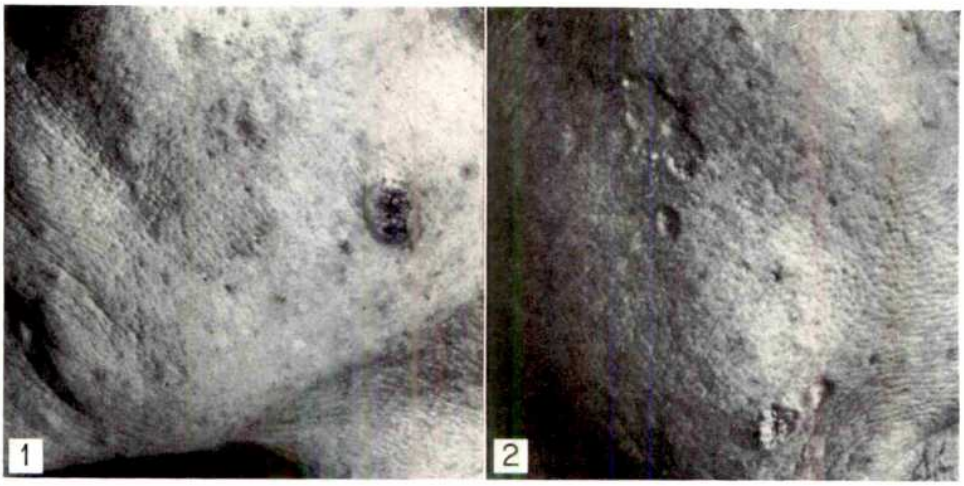


FIGURE 1. Keratoacanthoma on left cheek.

FIGURE 2. Crateriform scars on right cheek.

Investigations. Biopsies show typical features of a keratoacanthoma. Normal blood count, blood urea and liver function tests.

Treatment. There have been so many tumours that recently only those on the face have been curetted. Treatment with methotrexate is to be attempted.

(41) Nodular prurigo

DR P. HUDSON AND DR M.M. BLACK

St Thomas' Hospital, London

C.P., female, aged 31 years. Housewife. Hospital case no. 62 15 09.

History. She gives an 8-year history of nodules developing on her limbs. The first lesions appeared on her left foot and were described as being like insect bites. Gradually the lesions have spread, itching generally preceding the development of a nodule. Her general health is otherwise good.

Past history. She had hay fever as a child. In 1967 she was admitted to hospital for investigation of weight loss and abdominal pain for which no cause was found. In 1975 she had an anterior vaginal repair for stress incontinence.

On examination. There are numerous scattered nodules 0·5–1·0 cm diameter on her arms and legs; many of these nodules are excoriated (Fig. 1). There are a few nodules on each palm and on the soles. All other systems are clinically normal.

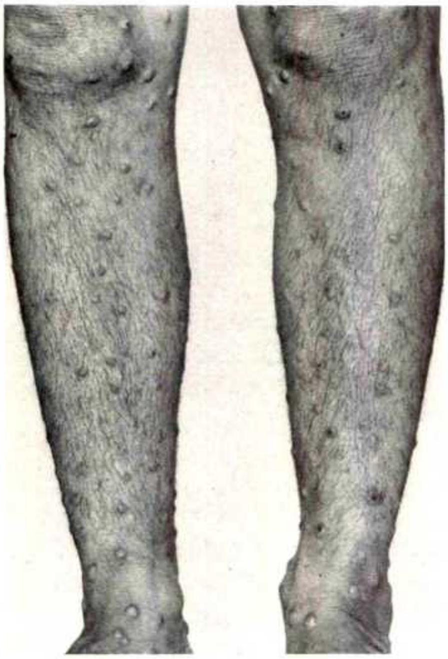
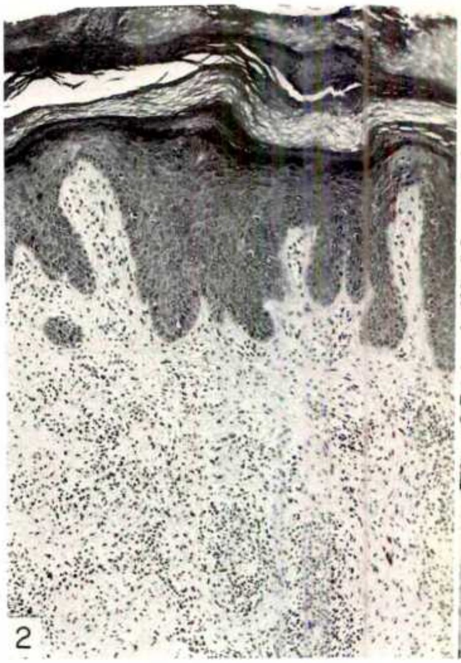


FIGURE 1. Nodules on legs.

Investigations. The following investigations are normal or negative: Full blood count and I serum iron and iron binding capacity, serum B_{12}, red cell folate, total proteins, calcium, phos alkaline phosphatase and jejunal biopsy.

Histology. Biopsy of a nodule showed typical changes of nodular prurigo (Fig. 2). Special with methylene blue showed a considerable excess of unmyelinated nerve fibres beneath the (Fig. 3).



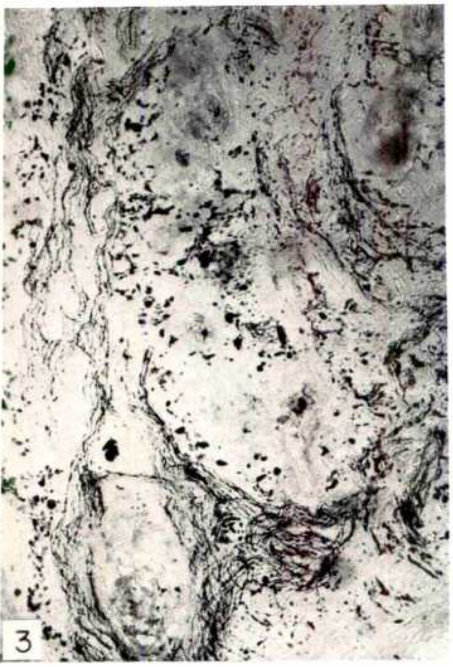


FIGURE 2. Biopsy of nodule (H & E stain).

FIGURE 3. Methylene blue stain showing hyperplasia and degeneration of nerve fibres.

Comment. This lady has classical nodular prurigo with typical lesions on her limbs and u lesions on her palms and soles. There are no stigmata of small bowel disease and investigati revealed no abnormality of the small bowel, although McKenzie (1975) describes three patien nodular prurigo and malabsorption, and Wells (1962) described one patient with nodular and adult coeliac disease. Another of our cases of nodular prurigo has hypolactasia and granulo hepatitis.

The methylene blue staining technique (Whimster, 1976) demonstrates hyperplasia and de tion of nerve fibres and a great increase in neural material, changes which have also been sh Cowan (1964) using the improved silver impregnation technique of Richardson.

Electron microscopy (Feuerman & Sandbank, 1974) has shown changes in the Schwann ce axons similar to those seen following irradiation damage. It is thought that the changes se therefore a non-specific reaction to injury, although it is by no means certain whether the changes are primary or secondary to the itching.

We are grateful to Dr Niels Jensen who kindly allowed us to investigate this patient.

REFERENCES

COWAN, M.A. (1964) Neurohistological changes in prurigo nodularis. *Archives of Dermatology*, **89**, 754.
FEUERMAN, E.J. & SANDBANK, M. (1975) Prurigo nodularis. *Archives of Dermatology*, **111**, 1472.
MCKENZIE, A.W. (1975) Personal communications.
WELLS, G.C. (1962) Skin disorders in relation to malabsorption. *British Medical Journal*, **ii**, 937.
WHIMSTER, I.W. (1976) To be published.

(42) Relapsing polychondritis

DR E.L. RHODES

St Helier Hospital

F.H., male, aged 80 years. Hospital case no. 26 39 86.

History and examination. Since September 1975 he has had three episodes of pain, swelling and redness of the right ear, accompanied by fever. This has left the right ear floppy and deformed (Fig. 1).

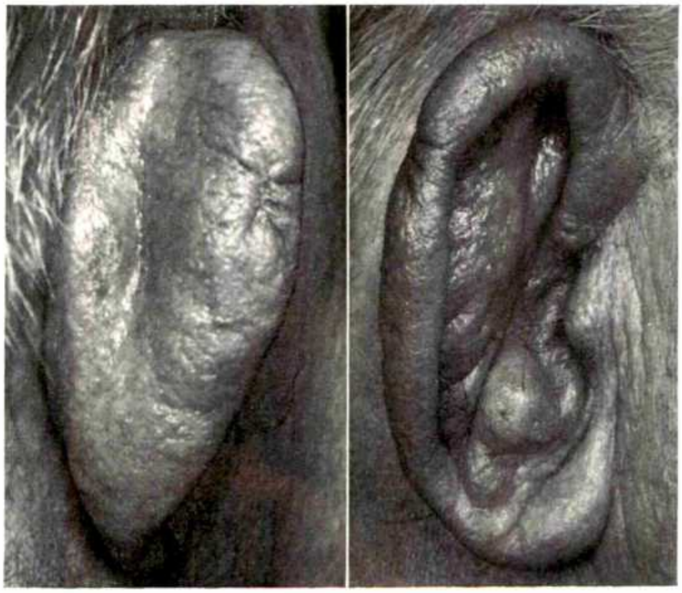


FIGURE 1. Right ear.

Investigations. Hb 11·7, WBC 13,800 (93% neutrophils), ESR 118 mm/1st h. W.R. negative. ANF negative. Serum anti-complementary activity positive. Chest X-ray, heart size normal, no pulmonary lesion seen. X-ray spine—appearances degenerative rather than those of ankylosing spondylitis. Mixed peripheral leukocyte migration inhibition (Dr Gange) showed inhibition to cartilage proteoglycans.

Treatment. The episodes of polychondritis have responded to short periods of steroid therapy.

(43) Management of stasis ulcers by pinch graft

D.A.Burns and I.Sarkany

Royal Free Hospital, London

C.H., female, aged 78 years.

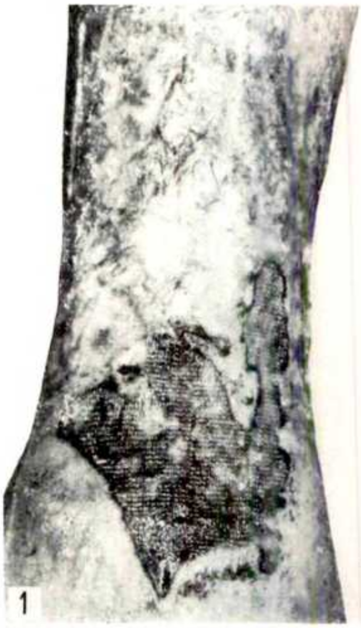
History. She has had recurrent stasis ulceration involving the medial aspect of both ankles for approximately 7 years. There are bilateral venous varicosities but no previous history of venous thrombosis. There is a family history of varicose veins.

Eight months ago the ulcers recurred and failed to respond to topical therapy.

Investigations. X-ray of legs: There is periostitis on the medial aspect of both tibiae and a little calcification in the soft tissues of the lower half of the leg, probably in relation to the varicose veins.

Patch tests positive to wool alcohols.

Management. Both ulcers were pinch grafted using the thigh as donor site. The recipient sites were dressed with paraffin gauze and left undisturbed for 6 days. At the end of this period dressings were removed and the grafted areas treated with Eusol paraffin soaks for 20 min twice daily until healed. Both areas were completely healed within 2 weeks of the grafting procedure (Figs 1 and 2).



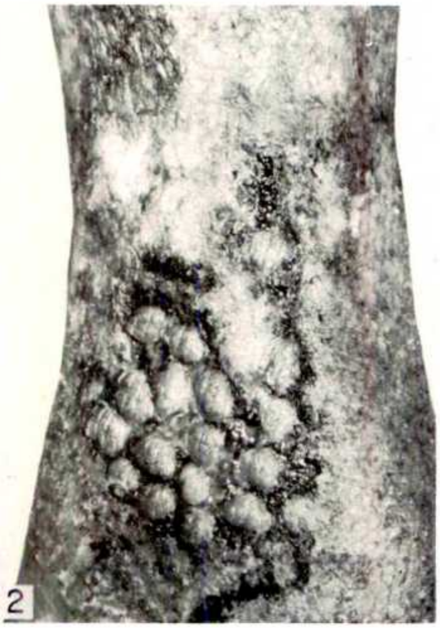


FIGURE 1. Stasis ulcer before grafting.

FIGURE 2. Stasis ulcer after pinch grafting.

Comment. This is one of twelve cases of stasis ulceration we have treated by this method. The procedure is carried out in the ward side-room under local anaesthesia with a minimum of discomfort to the patient. The donor site chosen is the lower abdomen or upper thigh. The recipient sites are cleaned with Eusol/paraffin solution for a few days prior to grafting, but in some cases grafting has been undertaken on heavily infected recipient sites, with good results.

The main advantages of this procedure are its simplicity and a reduction of duration of the npatient stay from an average of 3 months to 4 weeks.

(44) Case for diagnosis

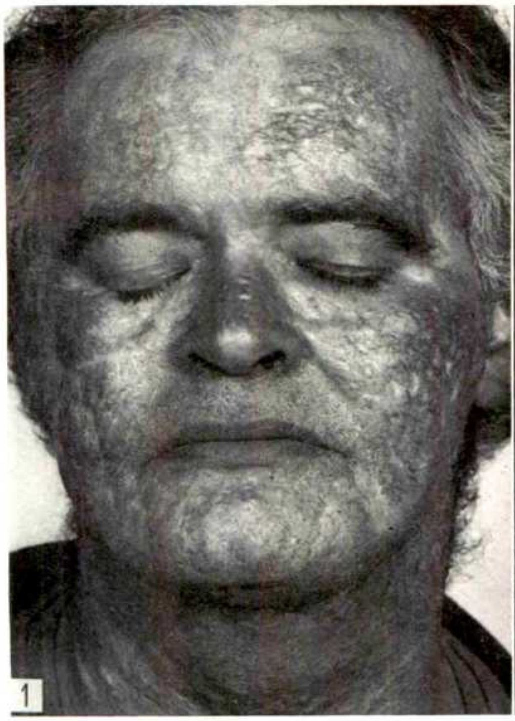
DR J.P.H.BYRNE FOR DR D.I.WILLIAMS

King's College Hospital, London

R.H., male, aged 36 years. Packer. Hospital case no. 10 50 73.

History. The patient first attended the Skin Department in 1951, and gave a history that since the age of 3 he had suffered from repeated episodes of inflammatory, ulcerating lesions on the face, buttocks, upper back and limbs necessitating years in hospital. Ulceration of these lesions was prominent but the ulcers healed leaving atrophic scarring. The scarring on the face was hypertrophic leading to ectropion of the right lower eyelid, correction of which has necessitated four plastic surgery operations. The patient suffered an episode of balanitis in 1972 which cleared rapidly following circumcision.

Examination. The patient looks old for his age and the face exhibits severe telangiectasia as a result of steroid therapy and also hypertrophic scarring (Fig. 1). On the limbs and upper back there are large areas of atrophic telangiectatic scarring, the end result of the inflammatory lesions. In some areas, especially the thighs, the edges of the scars are fimbriated. On the right leg are two erythematous papillomatous, vegetating lesions (Fig. 2). The legs show prominent follicular hyperkeratosis.



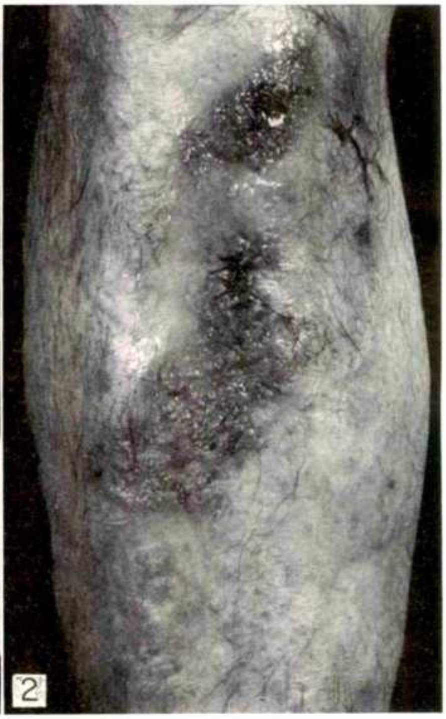


FIGURE 1. Photograph showing severe telangiectasia and hypertrophic scarring affecting the face.
FIGURE 2. Photograph of right leg showing erythematous papillomatous vegetating plaques.

Investigations. The haemoglobin is 14·0 g/dl, and the white cell count 13,600 cells/μl, with 84% polymorphonuclears. The erythrocyte sedimentation rate varies from 46 to 51 mm/h.

The total globulin level was 7·5 g/l with a rise in α_2 globulins and gamma globulins.

Immunoelectrophoresis was normal as was a biochemical (SMA) screen. An auto-antibody screen was negative. The Tine test was weakly positive.

Swabs from the boils and ulcers have consistently grown *Staphylococcus aureus* and *Streptococcus pyogenes*. Histology of a biopsy from a lesion on the right shin revealed patchy acanthosis and parakeratosis with a small, fairly chronic ulcer and occasional intra-epidermal microabscesses. The dermis also contains microabscesses and there is a diffuse, but not very heavy, chronic inflammatory infiltration with numerous plasma cells and some fibrosis.

Treatment. This patient was thought to have a sensitivity to his own Staphylococcal flora and was treated with Staphylococcal vaccines but to no effect. Whilst topical antibiotics especially gramicidin and neomycin (Graneodin) have helped, the most effective treatment has been systemic steroids. They have been used since 1966, the initial dose being prednisolone 40 mg per day. At present the patient is receiving 20 mg/day and Graneodin ointment topically.

Comment. No diagnosis has been made, although some form of vasculitis has been often considered.

(Contents continued from back cover)

Lipodystrophia Centrifugalis Abdominalis Infantilis; R. J. CAIRNS 44
Lichen Aureus; J. R. S. RENDALL FOR T. ROBINSON 45
Xeroderma Pigmentosum in Brother and Sister; M. KLABER AND J. S. PEGUM 47
Lipoid Proteinosis; N. SMITH FOR K. V. SANDERSON 48
Generalized Plane Xanthoma; M. P. JAMES AND S. C. GOLD 50
Lichen Myxoedematosus; J. P. H. BYRNE FOR R. H. MARTEN 52
Bisalbuminaemia; J. P. H. BYRNE FOR D. I. WILLIAMS 54
Sarcoidosis; D. I. VOLLUM 56
Cutaneous Ectopic Schistosomiasis; D. M. MACDONALD AND J. G. L. MORRISON FOR M. W. GREAVES 57
Acnitis; S. PEIRIS FOR D. I. WILLIAMS 58
Lupoid Sycosis; J. KIRBY AND D. D. MUNRO 59
Benign Hyperglobulinaemic Purpura of Waldenstrom; R. CLAYTON FOR M. FEIWEL 60
Livedo Reticularis with Thrombocythaemia in Association with Polycythaemia Rubra Vera; J. CLARKE AND G. C. WELLS 63
Painful Bruising Syndrome with Vasculitis Treated by Plasmapheresis; C. KENNEDY, K. V. SANDERSON AND C. SPRY 65
Pyoderma Gangrenosum with Cellular Immunity Deficit Treated with Plasmapheresis and Leukocyte transfusion; R. CLAYTON FOR M. FEIWEL AND H. VALDIMARSSON 67
Pyoderma Gangrenosum, Hypoplastic Anaemia, Tabes Dorsalis; D. I. VOLLUM 69
Chronic Intravascular Coagulopathy with ? Pyoderma Gangrenosum; R. C. D. STAUGHTON FOR P. W. M. COPEMAN 70
Chronic Granulomatous Disease; J. A. CARRUTHERS AND M. W. GREAVES 72
Erythroderma and Vasculitis; D. I. VOLLUM 74
Sézary Syndrome; R. C. D. STAUGHTON FOR P. D. SAMMAN AND D. E. SHARVILL 75
Bathing-trunk Naevus with Neoplastic Transformation; D. D. MUNRO 77
Multiple Keratoacanthomas; D. I. VOLLUM 78
Nodular Prurigo; P. HUDSON AND M. M. BLACK 79
Relapsing Polychondritis; E. L. RHODES 81
Management of Stasis Ulcers by Pinch Graft; D. A. BURNS AND I. SARKANY 82
Case for Diagnosis; J. P. H. BYRNE FOR D. I. WILLIAMS 83

British Journal of Dermatology, Volume 95, Supplement 14, July 1976

CONTENTS

PAGE

Foreword 1

Programme of the Meeting 5

Summaries of Papers

Nerve Supply as a Stimulator of the Growth of Tissues, Including Skin; I. W. WHIMSTER 9

Enzyme Cytochemical and Immunocytological Characterization of Cutaneous Lymphomas; O. BRAUN-FALCO AND G. BURG 9

Mycosis Fungoides with Leukaemic Transformation; CHRISTINE I. HARRINGTON 10

Trimethylaminuria or Fish Odour Syndrome in a Child; ROBIN MARKS, M. W. GREAVES, D. DANKS AND V. PLUMMER 11

Measurement of Dermal Atrophy Induced by Topical Steroids Using a Radiographic Technique; M. P. JAMES, M. M. BLACK AND C. G. SPARKES 12

Pseudopyogenic Granuloma: the Histopathogenesis in the Light of Ultrastructural Studies; R. A. J. EADY, T. COWEN AND E. WILSON JONES 13

Malignant Melanoma. A Comparison of Cellular Fine Structure in Invasive Nodules of Different Histogenic Type; J. A. A. HUNTER, S. ZAYNOUN, W. D. PATTERSON, R. M. MACKIE, A. J. COCHRAN AND S. S. BLEEHEN 13

Lentigo-maligna Melanoma: an Ultrastructural Study; S. S. BLEEHEN, LISA HARTLEY AND JENNIFER SENIOR 14

Cutaneous Tuberculosis of the Nose with Unusual Clinical and Histological Features Leading to Delay in Diagnosis; A. P. WARIN AND E. WILSON JONES 15

The Production of Antibiotics by Trichophyton and Epidermophyton Species; W. C. NOBLE, YVONNE M. CLAYTON, NAGWAN YOUSSEF, G. HOLT AND C. WYBORN 16

Taxonomy of Aerobic Cutaneous Diphtheroids; D. G. PITCHER 17

A Non-acne Naevus. A Clinical and Laboratory Study; W. J. CUNLIFFE, M. B. HODGKINS AND R. EAD 18

Pathology of Lichen Striatus; W. M. STEWART 18

Further Studies in Herpes Gestationis; J. A. CARRUTHERS AND M. M. BLACK 19

Digital Ischaemia due to Vascular Anomalies; NEVILLE R. ROWELL 20

Photochemotherapy. A Study of its Efficacy in Fifty Patients Suffering from Psoriasis and other Dermatoses; T. LAKSMIPATHI, P. GOULD, B. E. JOHNSON, L. A. MACKENZIE AND W. FRAIN-BELL 20

Effects and Side-effects of 8-methoxypsoralen-blacklight therapy in more than 1500 Patients; GERHARD WEBER 21

Increase in Prostaglandin E_2 and $F_{2\alpha}$ in Inflammatory Exudate at 4–48 h after UVB Irradiation of Human Skin; A. KOBZA BLACK, M. W. GREAVES, C. N. HENSBY, A. PAZZAGLIA AND N. PLUMMER 21

Case Reports

Juvenile Bullous Pemphigoid; M. SKEETE AND M. W. GREAVES 23

Transient Acantholytic Dermatosis; C. B. BENTLEY-PHILLIPS AND G. C. WELLS 25

Localized Pemphigoid; G. P. SPARROW AND E. J. MOYNAHAN 26

Porphyria Cutanea Tarda; M. KLABER, H. BAKER AND A. WARIN 28

Late Onset Erythropoietic Uroporphyria; A. MACDONALD, D. NICHOLSON AND R. WILLIAMS 30

Generalized Morphoea with Bullous Lesions and Psoriasis; D. A. BURNS AND I. SARKANY 33

Acute Widespread Morphoea; M. P. JAMES AND P. F. D. NAYLOR 34

? Scleroedema of Busche ? Eosinophilic Fasciitis; D. J. ATHERTON FOR R. S. WELLS 36

Cornelia de Lange Syndrome; D. M. MACDONALD FOR M. W. GREAVES 37

The Tricho-Rhino-Phalangeal Syndrome (a Family with Three Affected Members); F. BESSER FOR R. S. WELLS 39

Juvenile Dermatomyositis; E. B. D. HAMILTON 41

Piebaldism; D. R. TAYLOR FOR T. ROBINSON 43

(*Contents continued on inside back cover*)